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The microphonic effect of the lateral line organ

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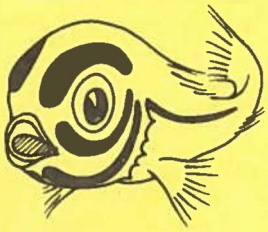
THE MICROPHONIC EFFECT OF THE LATERAL LINE ORGAN

A STUDY ON THE BIOPHYSICS AND THE FUNCTION OF THE LATERAL LINE ORGAN OF ACERINA CERNUA L.



JAN W. KUIPER

Laboratorium voor
Farmacognosie en Galenische Farmacie
Groningen

RIJKSUNIVERSITEIT TE GRONINGEN

THE MICROPHONIC EFFECT OF THE LATERAL LINE ORGAN

A STUDY ON THE BIOPHYSICS AND THE FUNCTION OF THE LATERAL LINE ORGAN OF ACERINA CERNUA L.

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN
DOCTOR IN DE WIS- EN NATUURKUNDE
AAN DE RIJKSUNIVERSITEIT TE GRONINGEN
OP GEZAG VAN DE RECTOR MAGNIFICUS
DR. J. ARIENS KAPPERS, HOOGLERAAR IN DE
FACULTEIT DER GENEESKUNDE, IN HET
OPENBAAR TE VERDEDIGEN OP
DINSDAG 6 NOVEMBER 1956
DES NAMIDDAGS OM 4 UUR

DOOR

JAN WILLEM KUIPER
GEBOREN TE HEERHUGOWAARD

UITGEVERIJ EXCELSIOR - ORANJEPLEIN 96 - 'S-GRAVENHAGE

Promotor: Prof. Dr. Hl. de Vries

STELLINGEN

1. De uitsluitend op gedragstudie gebaseerde opvatting dat één ommatidium de functionele eenheid van het Arthropoden oog is, moet als onjuist worden beschouwd.

zie De Vries, Hl. 1956 Progress of Biophysics 6
Waterman, T.H. 1954 Proc.Nat.Ac.Sc. 40

2. Voor het compenseren van de parasitaire ingangscapaciteit, welke optreedt bij het gebruik van micro-electroden, stelt Woodbury een electronische schakeling voor waarvan het principe goed is maar door hem op een onjuiste wijze wordt uitgevoerd.

Woodbury, 1952. J.Cell.Comp Physiol. 39

3. Het is niet waarschijnlijk dat het mechanisme met behulp waarvan insecten de polarisatiegraad van gepolariseerd licht bepalen, berust op reflectie aan lensoppervlakken.

zie o.a. Waterman, T.H. 1954. Proc.Nat.Ac.Sc. 40

4. De op het diluvium van West Europa voorkomende heide mag niet beschouwd worden als een glaciaal relict.

5. Doordat men een verkeerde waarde voor het getal van Avogadro heeft gebruikt, liggen de door Karrer opgegeven drempelwaarden voor de reuk een factor 100 te laag.

Karrer, P. 1950. Organic Chemistry tabel XIV p.933

6. Om de selectiviteit in de ionenopname van planten te kunnen verklaren moet het door Lundegårdh voorgestelde anionen ademhalingsmechanisme uitgebreid worden.

Lundegårdh, H. 1954. Symposia S.E.B.VIII.

7. De door Sakai bepaalde eigenschappen (transmissie en scheidend vermogen) van een „spiral-orbit" spectrometer zijn niet maatgevend voor de kwaliteit van deze spectrometer als kernphysisch meetinstrument. De wijze, waarop Siegbahn Gerholm deze spectrometer met andere vergelijken is daarom misleidend.

Sakai, M. 1950. J.Phys.Soc.Japan 5: 178
Siegbahn, K. 1955. Beta- en Gamma-ray Spectroscopy, p.75.
Gerholm, T.R. 1956. Handb.d.Phys. XXXII, 0.628.

8. Het zijlijnorgaan moet opgevat worden als een tastorgaan.

9. Het is waarschijnlijk, dat ook bij het ontstaan van het microphonisch effect van de labyrinth organen acetylcholine een essentiële rol speelt.

10. Het is nog niet gelukt een physico-chemische basis te geven aan het biologisch zeer bruikbare gen-begrip.

Goldschmidt, R.B. 1955. Theoretical Genetics.

11. Op grond van de ontwikkeling van de experimentele biologie verdient het overweging om voor studenten in deze vakken dezelfde mathematische opleiding verplicht te stellen als thans verlangd wordt van de studenten in de chemie

12. Bij de bouw en de inrichting van Universitaire Laboratoria moet men meer gebruik maken van de functionele kleurenleer.

Aan mijn Ouders
Aan mijn Gezin

The experiments reported in this thesis have been financially supported by the Netherlands Organization for Pure Research (Z. W. O.).

This publication has been made possible by a grant of the Netherlands Ministry of Education, Arts and Sciences (ministerie van Onderwijs, Kunsten en Wetenschappen.).

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Bij het voltooien van dit proefschrift wil ik een woord van dank richten tot allen die tot mijn wetenschappelijke vorming als bioloog hebben bijgedragen.

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Hoewel mijn belangstelling vooral op zuiverwetenschappelijk onderzoek gericht is, prijs ik mij gelukkig een aantal jaren toegepast biologisch onderzoek te hebben mogen doen. Moge ik op deze plaats mijn dank uitspreken jegens het Instituut Veeteeltkundig Onderzoek T.N.O. te Utrecht en het Rijksinstituut voor Pluimveeteelt te Beekbergen en mijn vroegere collegae bedanken voor hetgeen zij voor mij hebben gedaan.

Het valt mij moeilijk mijn dankbaarheid jegens U, hooggeleerde de Vries, mijn promotor, onder woorden te brengen. U hebt mij een door U begonnen onderzoek laten voort zetten en mij daarbij een complete apparatuur ter beschikking gesteld. Tijdens de experimentele phase van het onderzoek hebben de korte discussies met U sterk stimulerend gewerkt. Tenslotte hebt U mij in de gelegenheid gesteld Uw kritiek in dit proefschrift te verwerken. Mijn dankbaarheid jegens U is dan ook groot en, hoewel U voor mij geen gemakkelijk leermeester bent, acht ik het een voorrecht Uw leerling en naaste medewerker te zijn.

Hooggeleerde Brinkman, dat ik mij als bioloog geheel thuis voel op het Natuurkundig Laboratorium is grotendeels te danken aan de wijze waarop U het directoraat hierover voert. Ik zeg in Uw persoon de gehele wetenschappelijke en technische staf dank voor alle hulp die ik heb ondervonden.

Hooggeleerde Huizinga, de mogelijkheden die U de biophysici verschaft op Uw kliniek zijn van grote waarde. In het bijzonder zeg ik U hierbij dank voor de faciliteiten die U mij persoonlijk verstrekt heeft.

Hooggeleerde Gaarenstroom, hooggeleerde Keuning, voor de hulp die U mij heeft gegeven ben ik U zeer erkentelijk.

In de loop van het onderzoek heb ik verschillende tijdelijke medewerkers gehad. Renske Jielof, A. Spoor, J. Emck, M. Stuiver, J. Balkema (physici), J. Mook en P. R. Wiepkema (biologen) en dr. D. Ubbens (medicus) mijn hartelijke dank voor jullie hulp en steun. Truus van Zanten dat ik jou ongeveer tot

mijn vaste medewerkster mag rekenen stel ik zeer op prijs.

Bij het prepareren van de Engelse tekst heb ik kunnen samenwerken met mijn vriendin, wijlen mevrouw Magda Lenters-Eibergen. Dit proefschrift zal voor mij een goede herinnering zijn aan onze samenwerking

Mejuffrouw J. M. Willinge, de enthousiaste en vriendschappelijke wijze waarop U Magda's taak hebt overgenomen, verdient hier vermeld te worden.

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Chapter I

GENERAL OUTLINE OF THE PROBLEM

Physical and chemical agents are capable of changing the energetic situation in an organism in a distinct way, which is often demonstrated by the organism in the form of certain spontaneous reactions. When no reactions occur it is of course too naive to suppose that an animal does not perceive the stimulus. There is a possibility that an animal does not show a change in its behaviour though it perceives the stimulus because the stimulus has no biological meaning for it. In various cases it has been possible to interest an animal in a stimulus by giving it food during the stimulation. Then after some time it will connect the stimulus with the food and it will demonstrate after application of the stimulus only a typical behaviour search for food. From this behaviour we may conclude that the animal perceives the stimulus. With this method – conditioned reflex method – biologists have found out what animals can perceive with their sense organs. For example von Frisch and his coworkers discovered that fish can hear and that bees see ultraviolet light and are capable of detecting the degree of polarisation of light.

A physiologist, however, likes to know what happens in the sense organs when these are stimulated. He asks: What are the alterations induced by the stimulus? This is the basic question we are dealing with in this study.

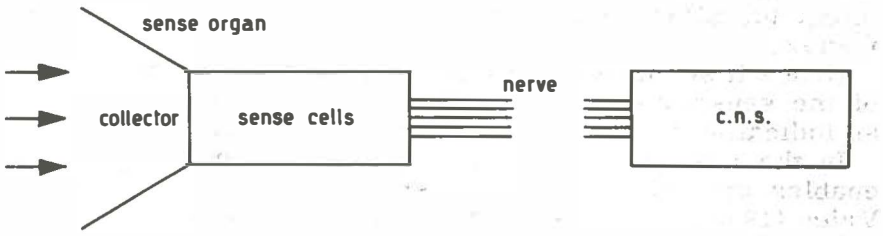


Figure 1. Diagram of a sensorium; sense organ with collector and detector unit (sense cells), the afferent nerve and the central nervous system.

Let us consider briefly the mechanism of a sensory system (sensorium). A diagram of such a system is given in figure 1. The collector concentrates the energy of the stimulus on the detector, i.e. on the sense cells. To begin with biophysicists studied the physical problems of the collector and for

instance much is known on the mechanics of the ear. The details concerning the mechanics of the collector system of the mechanical sense organs we are dealing with in this thesis are not yet known. But it is possible to go further and tackle the problem concerned with the induced change in the sense cells, which now logically presents itself.

At present not so very much is known about these changes. But well known are the properties of the signals in the afferent nerves coming from the sense organ. These are short electrical impulses, so-called "action potentials" or "nerve-spikes". The number of impulses passing per unit time depends on the quality and the quantity of the stimulus applied. This holds for all the sense organs investigated.

From this we may draw the conclusion that in the sense organs a process is taking place which results in the initiation of the action potentials in the afferent nerves. Since the nerve fibres in the entire animal world have physiologically more common features than differences, it is quite possible that this stimulating process of nerves may also be the same in different kinds of sense organs.

In this connection it is striking to note that in the best studied organs, the ear and the eye, electric changes do occur when these organs are stimulated. In either organ measurements have shown that the apical part of the sense cell becomes negative with respect to the basal part. So during stimulation of the sense organ there is an electric current in this organ. In addition it is a well known fact that activity can be easily initiated in a nerve by an electric current. Hence it is very attractive to postulate that the stimulation of the sense cells results in a current around these cells and that this current initiates the impulses in the nerve fibres near the sense cells. This hypothesis is indeed accepted by various sense physiologists, although direct proof of this is lacking as far as I know.

Hence it seems worth while to study this electrical activity of the sensory organs in detail. In addition it can be used as an indicator for the processes in the sense cells.

In the lateral line organ of a fish (a sense organ which enables the animal to detect small water displacements) de Vries (1948, 1952) discovered such an electrical effect. It is called the ***microphonic effect*** of the lateral line organ, by reason of a well known similar effect in the ear.
The greater part of this study deals with this electrical effect in the lateral line organ.

The central question is: How is this effect produced by the sense cells, or in other words, what is the change caused by the stimulus in the sense cells of this organ?

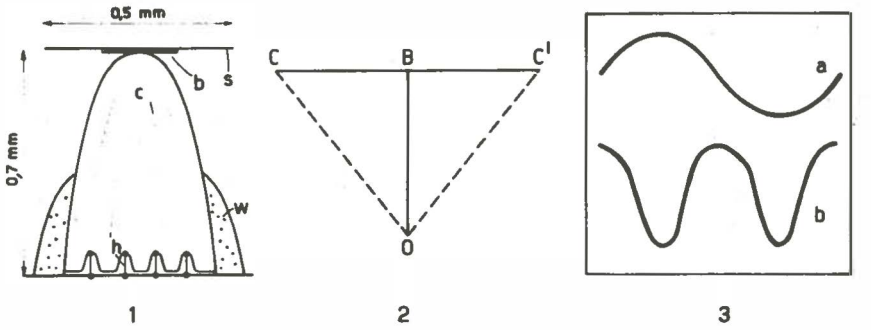


Figure 2. Schematic diagram of the lateral line organ in 1 with skin (s), bone bridge (b), cupula (c) with the surrounding wall (w). The hairs (h) on the sense cells (these are not drawn) are stretched twice each vibration period when the cupula slides from the right to the left and back. In 2 this stretching effect is indicated; CB corresponds with the amplitude of the cupula. In 3 by (a) the movement of the cupula is indicated and by (b) the observed microphonic effect.

A schematic picture of the organ is given in figure 2. It consists of a gelatinous structure, the cupula, resting on layer of sense cells. When the organ is stimulated by an alternating water current we measure in the vicinity an alternating current which is of the double frequency of the vibration applied. To account for this doubling of the frequency de Vries (1952) suggested that the cupula slides to and fro over the sense cells. When cellular protrusions, called hairs, are connected with the cupula as indicated in figure 2-1, these hairs are pulled twice each vibration period of the cupula. De Vries assumed that the voltage of the microphonic effect is related to this pulling of the hairs. For a more extensive discussion of this hair-hypothesis see chapter V.

In the hair-hypothesis it is assumed that hairs are situated on the sense cells. In chapter III we will deal with this existence of hairs.

The physics of the collector, especially the mechanics of the cupula is described in chapter V.

Since the microphonic effect of the lateral line organ is not only an interesting subject in itself, but as it is also related to the similar effect in the ear and the balancing organ of the vertebrates, a short survey on the literature of the electrical effect of these organs is given in chapter IV.

After a detailed discussion of the hair hypothesis of Professor de Vries, the experimental problems concerning the microphonic effect are formulated in chapter VI.

The quantitative experimental results are given in chapter VII. These results will then be discussed in chapter VIII in connection with some extensions and modifications of the original hair-hypothesis.

In the chapters that follow, the microphonic effect is approached from a more physiological point of view. We shall

deal a. o. with the part played by acethylcholine in the origin of the microphonic effect in chapter XI.

Chapter II is devoted to the principles of the techniques used in this investigation.

Some confusion exists in the literature on the function of the lateral line organ. In the electrophysiological experiments the organ demonstrates a considerable sensitivity to vibrations. However when trying to use the conditioned reflex method previous investigators have failed in the training of fishes to vibrations which the animals could only perceive with the lateral line organ. In the final chapter a succesful experiment is described in which it is shown that it is possible to train Acerina cernua with the help of vibrations applied only to this organ. The same species is also used in the electrophysiological investigations.

Chapter II

APPARATUS AND TECHNICAL QUESTIONS

The aim of this chapter is to give a description of the techniques used in this study. It is beyond the scope of this publication to give an extensive description of the apparatus. Moreover the greater part of the apparatus was finished when I started these investigations. It was built by de Vries and described by him (de Vries, Jielof and Spoor 1952).

Fish. With a few exceptions all the fish, ruffs (Acernina cernua), were collected in the "Zuidlaarder meer", a lake in the neighbourhood of Groningen. The animals were kept in aquaria with running water and fed with meat and worms. When collected in autumn and winter the mortality was about 10% during the first weeks of their stay in the laboratory. Animals collected in spring and summer had a high mortality, often more than 50%. When the animals survived the critical period and took food, which was not always the case, they could be kept alive a year. New animals were always startled by sudden movements near the aquarium, or when a lamp was turned on in the room. Sometimes the following remarkable behaviour could be observed. The animal moves very quickly through the water and bumps against the glass wall, it stiffens, the gill covers move sideways and the spiny backfin is raised. Not a single respiratory movement is seen in the first minute, then it starts irregularly. After about a quarter of an hour the animal usually swims normally through the aquarium. I gaines the impression that the fish does not survive a third or fourth "convulsion". This may be one of the reasons of the high mortality during the first weeks. Perch (Perca fluviatilis) sometimes show similar convulsions.

The mortality during the experiments was low. The animals easily survived an ethylurethan narcosis. The operation on the lateral line organ is small and I never had an indication that the animals died as a consequence of this. As most of the other experimentators with fish I never observed a wound infection.

Fish-holder. The animal was tied to a glass strip and a conical glass tube was placed in its mouth. This tube prevented it from slipping forward and moreover served as a water supply for the artificial respiration. Fish-holder and fish were put in a perspex tank mounted on a 2 cm thick iron plate. The respiration movements are accompanied by elec-

trical fluctuations and these are troublesome in the electrical measurements. Moreover the right gillcover and the lower jaw are usually fixed against the holder and when the fish tries to move these the whole head is moving. These movements are very inconvenient when measuring the amplitude of the cupula. When the respiration water had been saturated with oxygen the frequency of the movements diminished and did not interfere with measurements.

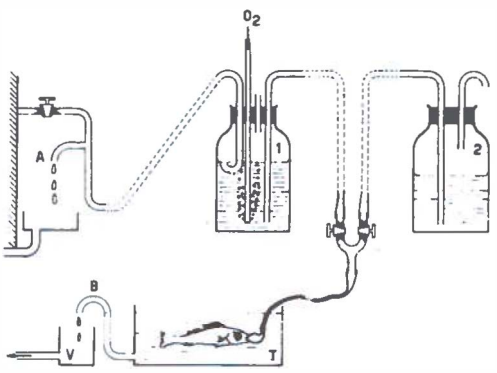


Figure 3. Artificial respiration arrangement. An overflow keeping the water level in vessel 1 at a constant height; oxygen through 1; 2 contains narcotic fluid; T, fishtank, the level of the water in this is kept constant by overflow B; the water in V is removed by a water-jet-pump.

In figure 3 the water supply for the artificial respiration is indicated. The level of the water in bottle 1 was kept constant with the aid of the overflow A, linked to the tap of the water supply of the laboratory. Through the bottle oxygen bubbled. The fluid was brought to the fish by means of a siphon. A second overflow B kept the water level in the fishtank T constant. The water was removed from V with a waterjet pump: The second bottle in figure 3 was used for the narcotic, 0,5% ethyluretan in tap water.

Stand. To get a mechanically stable framing de Vries constructed a special stand. This has proved to be very useful in various types of physiological experiments. It consists of a heavy iron plate 40x40 cm^2, the upper part of which is polished. The fish-tank could be moved smoothly over this foundation plate greased with lanoline. To a bar fitted to this plate, smaller ones could be coupled and to these various instruments may be connected.

Microscope. A stereo-microscope, an ordinary dissecting microscope, was connected to the stand. All the experiments were made under optical control with the aid of this micro-

scope; usually a 10 x magnification was sufficient. During the measurements of the amplitude of the cupula the stereo-microscope was replaced by an ordinary microscope provided with a water immersion objective. The magnification of this was 600 x; a distance of 1 micron could be observed.

Manipulators. In most of the investigations simple ball manipulators were used. They consist of a steel ball on which a bar was solded and on the end of this the electrode wire was connected. The ball fits in a cup and this could be moved along a platform which was mounted on the same iron plate as the water tank. The movable parts were lubricated with lanoline.

In later experiments a micromanipulator was needed. Its principle is described by von Békésy 1952, and we have called it the "Békésy manipulator". The principle is indicated in figure 4. 0 is the centre of the curvature and is connected with B. If B is shifted to B', then A moves to A' and the diminution depends on BO/AO. When A is on the other side of O the movement is reversed.

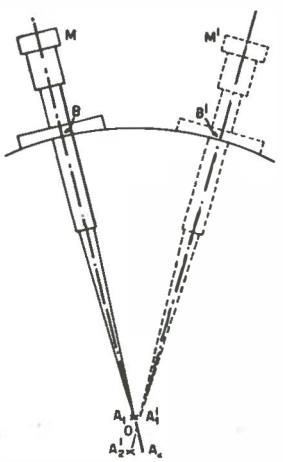


Figure 4. BB' part of a circle with centre 0. When the manipulator is moved from M to M' the tip A is displaced from A1 to A1'. When the tip is on the other side of 0 the movement is reversed.

According to this idea we constructed a micromanipulator. (fig. 5). Our manipulator differs from the original one by von Békésy in this respect that the latter's manipulator, used as an operative tool, creeps back to a medium position, whereas our instrument remains in the position in which we put it. Moreover a micrometerscrew made it possible to move the tip of the electrode over a distinct range.

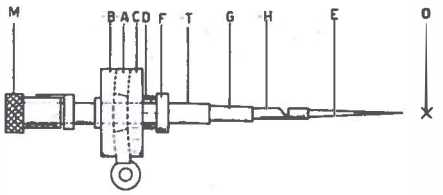


Figure 5. Békésy manipulator. A is a fixed and perforated convex-concave lens, O is the centre of curvature. B is connected to tube T; C is pressed against A by spring D which can be adjusted by screw F. M is a micrometerscrew by which plunger G and the electrode holder H can be moved in a vertical direction.

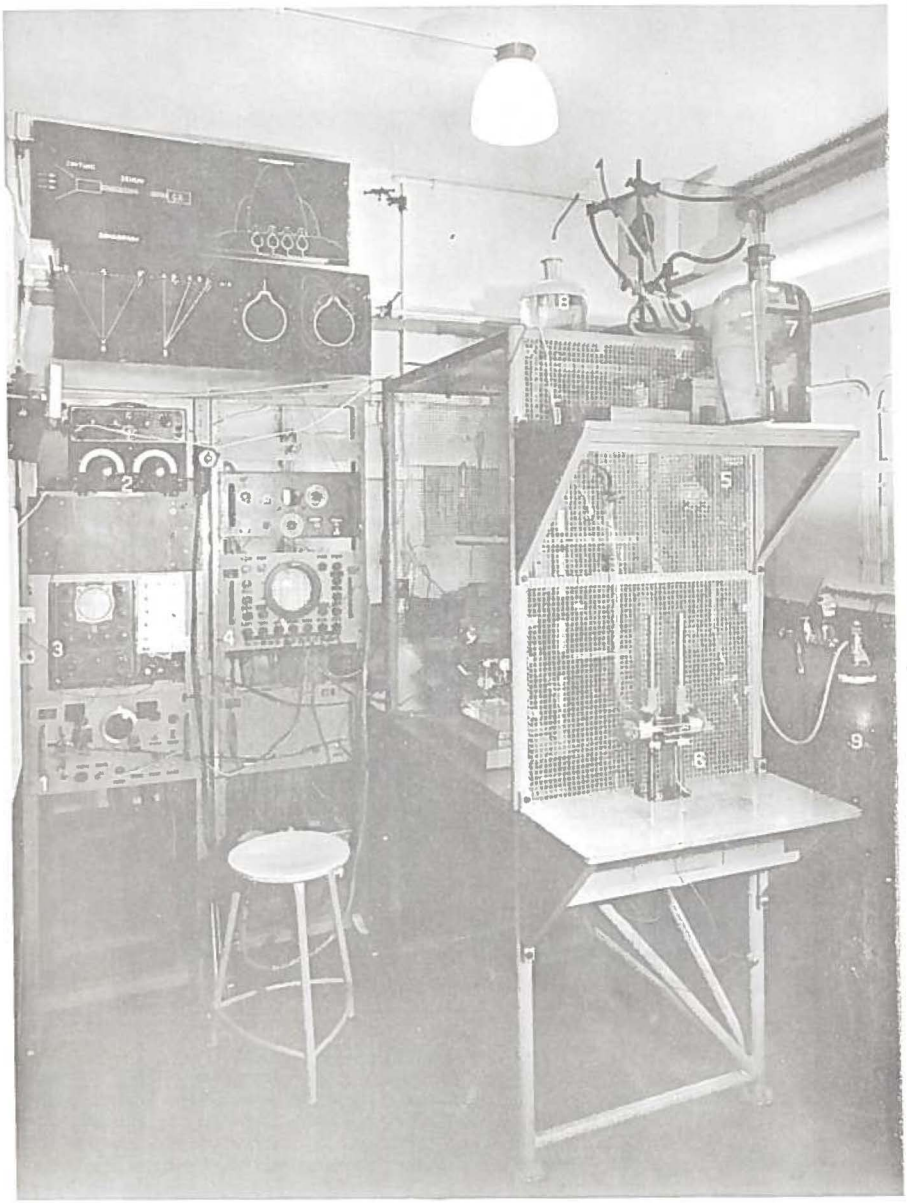
At both sides of a fixed and perforated convex-concave ground steel lens (A) two exactly fitting brass lenses are connected (B and C). To the upper one the tube of the micrometer is fixed, whereas the other one is pressed against the central part by spring D. By turning the micrometerscrew (M) the tip of the micro-electrode moves in the direction of the radius of the curvature of the lenses. The other directions were obtained by moving the entire movable part along the fixed piece. This manipulator was mounted on a bar on the iron plate to which the perspex watertank was connected.

The advantage of this type of micromanipulator is that it is rather small and especially when the exact shifting in only one direction is desired it is a handy instrument.

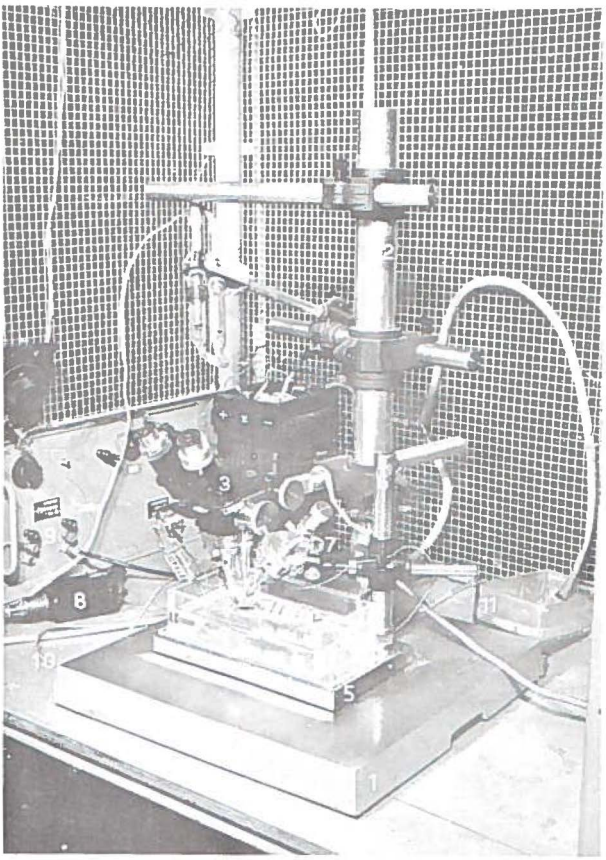
The fish with manipulators and the driving coil were all connected to the same ironplate. This could slide over the foundation plate of the stand and so be shifted under the microscope without changing the experimental situation.

Stimulating devices. During the experiments described on page 77 I used at first the same driving method as de Vries c.s. used. Alternating water currents were obtained with the aid of an electro-magnetic tuning fork on which a small pump was coupled and this was connected with a glass tube, filled with water. With this system we were restricted to the frequencies 11, 14, 22, 37, 44, 60 and 88 c.p.s. (resonance frequencies). Sometimes a second high frequency was applied by pressing the moving system of a loudspeaker against the glass tube which provided the cupula with the alternating water currents.

In most of the experiments described here a magnetic driving method was used. It was conceived by de Vries. In essence this is a very small piece of magnetic material (Ticonal) which should be mounted on the cupula and driven by an electromagnet. In a homogeneous field the magnet will not move when its axis is parallel to the lines of force; if not, there will be a couple (fig. 6). In the inhomogeneous field of



Photograph 1. The apparatus used in this study. (1) and (2) are a.c.-generators. Oscilloscope (3) is used for the control of the input. The microphonic effect is studied with oscilloscope (4). (5) is the Faraday screen around the experimental arrangement on the table. (6) is the tunning fork with which alternating water movements were produced. Bottle (7) contains the respiration fluid through which oxygen bubbles from (9). Bottle (8) contains the narcotic fluid.



Photograph 2. The experimental equipment. Along the polished ironplate (1) the fishtank (5) can be moved. To the bar (2) the microscope (3) and the strobascope lamp (4) are connected. The Békésy manipulator (7) is connected to bar 6 which is connected to 5. The dissecting microscope (3) can be replaced by an ordinary monocular microscope (8) supplied with a water-immersion objective. Number (9) indicates the front panel of the pre-amplifier. (10) if the tube of the water supply to the fish. (11) the overflow to keep the waterlevel in the fish tank at a constant height.

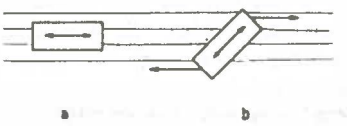


Figure 6. Magnet in a homogenous magnetic field. In situation a there is no resulting force; in b a couple results.

the more or less conically wound driving coil we used, the magnet was attracted and repulsed (see fig. 9). In some experiments in which the magnet was mounted on the skin the couple acting on the magnet was used (fig. 6b). The one end of the magnet was fixed on the skull and the other moved up and down.

To realise this driving method the first difficulty we met with arose from the dimensions of the lateral line organ. The organ does not exceed 0,5 mm, therefore the magnet must be of a similar size, preferably smaller.

The Philips works at Eindhoven kindly supplied non-magnetized ticonal wire with a cross section of 0,2 x 0,3 mm^2. With a pair of scissors small cubes were cut from this material, and these were magnetised between the poles of a strong permanent magnet. The magnetic moment of ticonal is high, so the magnets are rather strong. The small magnets were handled with a watchmaker's tweezers made of nonferromagnetic material; in this case new silver.

The second difficulty to be surmounted was the fixation of the magnet on the cupula. After various unsuccessful efforts the following way proved the best.

The non-directed ticonal pieces were solded on a silver foil and then cut out of the foil with a silverstrip on two sides. The strips were bent as indicated in figure 7. The small cap was polarised in such a way that the north-south axis was known.

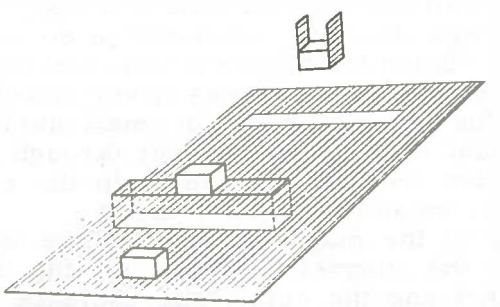


Figure 7. Different stages of the production of the "magnet caps". Magnet solded on the silver sheet, cut out with the strips, and the way the strips are bent.

The cap was placed on top of the cupula and the strips bent towards each other in order to fix the cap to the cupula. Sometimes the fixation was not so firm. In those cases the amplitude of the cap could be seen microscopically to exceed the amplitude of the cupula when the magnet was driven by the driving coil. In such cases the frequency characteristic of the microphonic effect (see page 67) has more than one peak; sometimes the frequency of the microphonic effect is four times the frequency of the current through the coil. When the amplitude of the cupula was determined, I always checked whether there was any difference between the cupula and the magnet in this respect. In many cases the magnet was mounted on the skin as indicated in figure 8.

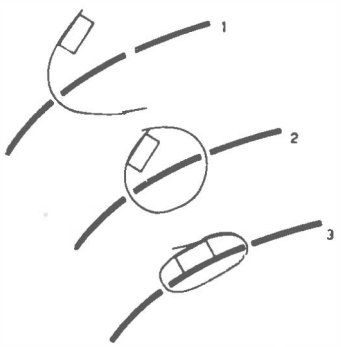


Figure 8. Fixation of the magnet on the skin of the lateral line organ. In three stages it is indicated how the strip is sewn through two small openings in the skin.

As the canal of the lateral line organ is small the driving coil must be likewise. It was wound under the microscope on a 0,1 mm thick and 1 mm long core at the end of a brass rod. First a mica sheet was cemented with Araldit at the end of the core and between this sheet and the 1 mm thick bar the coil was wound. The windings were cemented with Araldit. The data of the coil are: radius about 0.4 mm, length 2 mm, number of windings about 100, diameter of the wire 0.05 mm, The resistance varied for different coils and depended on the length of the lead-in wires; it was never more than 1 ohm, usually less. The self-inductance is small and it was proved that at a constant voltage the current through the coil was independent of the frequency, at least in the range used in the experiments, so up to about 500 c.p.s.

The intensity of the magnetic field of the coil, i.e. the force acting on the magnet, depends on the distance between the magnet and the coil. This distance was usually about 0,2 mm. The amplitude of the cupula in the experiments described here was not more than 15 microns and this has

approximately no influence on the force acting on the magnet. In this case the force is directly proportional to i and to V, the potential on the driving coil as measured on the screen of the oscillograph.

The force necessary to get a certain displacement of the cupula can be measured, but I have not done this.

Of course it was of great importance to make sure that the position of the coil in respect to the magnet did not alter during the measurements. Therefore the situation was often checked under the microscope. A second point regularly checked was the relation between the input (the voltage on the coil) and the output (the voltage of the microphonic effect) at a certain frequency. In the beginning the coil often moved either because I lacked experience and sometimes touched parts of the apparatus or because the manipulator had back lash. When these physical troubles were conquered there remained some biological difficulties. The most important one was that after some time (1 - 2 hours), the microphonic effect decreased so that the measurements had to be carried out within this time.

Recording devices. Most of these are built under the supervision of Prof. Hl. de Vries in the laboratory. They are of the conventional design as used by many electro-physiologists and I think they need no detailed description.

The apparatus used during the first experiments (on the superposition effect see chapter VII page 67) was the same as used by de Vries, Jielof and Spoor (1952). It consists of a simple RC-amplifier and oscilloscope, both for low frequencies. The constancy of the amplification was checked and no phase shifts occurred at the frequencies studied. In the other experiments I used an oscilloscope supplied with a differential amplifier, which was later on converted to a direct current amplifier. The signal was put direct on to the grid of the first tube and led to the oscilloscope amplifier through a cathode follower.

The oscilloscope had a double beam tube; the output signal of the fish was put to one channel and the voltage on the coil to the other. In this way it was very easy to measure the phase behaviour of the microphonic effect. The oscilloscope was synchronised by the same AC-generator feeding the stroboscopelamp and the driving coil. See block diagram in figure 9.

For the measurements the output of the lateral line organ was synchronised. Due to the irregular movements of the fish the subsequent curves did not exactly coincide and therefore the output-curve was not as sharp as indicated in figure 9.

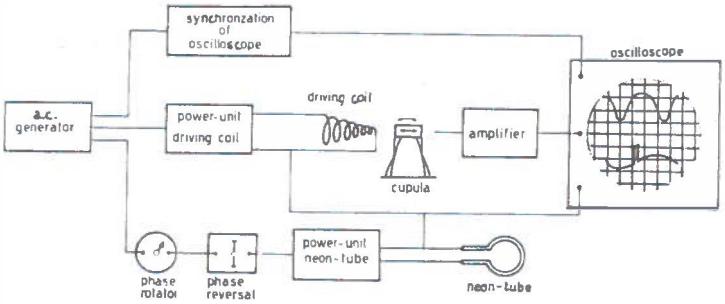


Figure 9. Block-diagram of the driving and recording apparatus.

To the precise study of the wave form the oscilloscope was supplied with a special brightening circuit. After the signal was synchronised the intensity of the beam was suppressed. Then the brightening circuit was closed with the flash contact of the camera and the beam was brightened during the next full period of the sweep, from the left to the right.

In the experiments concerning the d.c.-component of the microphonic effect the driving system was started by the same trigger as the time base. A delay-time was introduced by a relay closing the driving circuit and so in each recording a "zero line" was first recorded. A similar system was used in the investigations on the effect of constant displacements of the cupula; then a direct current was led through the coil. In the c.s. experiments I used as pick-up electrodes non-polarisable electrodes of the type Cu, $CuSO_4$ saturated solution, KCl (gelatin solution). Usually this electrode was glass capillary with a tip diameter of about 0,2 mm. In some experiments micro-electrodes were used with a tip diameter smaller than 1 micron. These were made in a home-made "microforge" of the type described by Fonbrune.

Details of the technique, the filling with an electrolyte solution and the electric properties of these electrodes will be published elsewhere.

Stroboscopic illumination. To inspect the motion of the cupula a stroboscopic illumination is used. This is described by de Vries et al. 1952. A block diagram of the whole driving-, recording- and illumination-apparatus is given in figure 9. One a.c.-generator feeds all devices, which means that all parts have the same frequency and a constant phase relation.

The stroboscopic lamp was mounted to the stand. It was a neon-tube bent in a ring shape and this ring fits around the microscope objective. In this way an illumination from all sides was obtained.

On the sine-wave indicating the voltage on the driving coil, a peak marks the moment of the light flash (see fig. 9). With

the phase rotator the observer can continuously adjust the moment of the light flash. With the "reversal" switch it was possible to shift this moment abruptly over half a period. The duration of the flash can be altered from 0,1 to 5 milliseconds.

Suppose the moment of the light flash in figure 9 corresponds to the moment in which the vibrating object, in casu the cupula of the lateral line organ, passes the equilibrium position. When the light comes half a period sooner, then the cupula is seen on the same place in the microscopic field.

When turning the switch quickly no displacement can be observed if the moment of the flash really corresponds to the equilibrium position of the cupula, whereas in all other cases there is a displacement. In this way the phase behaviour of the cupula was determined in respect to the voltage on the driving coil. By comparing it with the phase of the microphonic effect as to the same voltage curve, the difference in phase between the mechanical and the electrical response of the organ was calculated. The possible error of this value does not exceed 10^{o} and mainly arises from the mechanical measurements.

The amplitude of the cupula was measured in the following way. After adjusting the moment of the light flash so that it illuminated the cupula in its equilibrium position, the moment of the light flash was altered over a quarter of the vibration period. Then the cupula is illuminated in an extreme position. When now the moment of the light flash was changed over half a vibration period the other extreme position is illuminated. Half of the displacement observed in this way corresponds to the amplitude of the organ. The error in these measurements is of the order of one micron.

The histological technique was the wellknown paraffin method. Fixation was done with Bouin's fluid; when the structure of the cupula itself was studied the fluid had a pH 7. When the entire head was used the skull was also decalcified in Bouin. The nerves were stained with the Bodian technique, but I think the Bielshowsky method will give much better results, especially as far as the arborisation at the sense cells is concerned. Some preparations were made with the freeze-drying technique. The object, a single lateral line organ, was frozen in liquid propane kept fluid in liquid oxygen. It was dried in hydrogen atmosphere, pressure approximately 0,5 mm Hg. After drying the tissue was embedded in paraffin, also in vacuum.

After cutting the sections, the solid paraffin was removed with liquid paraffin which in its turn was removed by a mixture of castor-oil and -bromonaphtalene (refraction index for these mixtures ranging from 1.4 - 1.6). These preparations were studied with the phase-contrast-microscope and

the polarisation microscope. The preparations were not very good. The principal aim was to study the distribution of lipoid in the sense epithelium. It was possible to draw some preliminary conclusions from these preparations.

In some cases also freeze-microtome slides were made and studied with the polarisation microscope.

Chapter III

THE STRUCTURE OF THE LATERAL LINE ORGAN

This chapter has been written to provide an anatomical background for the electrophysiological studies in the next chapters. For the lateral line organ this is based on some histological investigations of the present author, and for the labyrinth organs on the literature on this organ. The chapter further contains some ideas on the origin of the gelatinous part of these sense organs. Special attention is paid to the hairs on the sense cells.

Introduction

The lateral line organ is found on Vertebrata (Cycyostomata, Elasmobranchia, Pisces and Amphibia) living in water. Macroscopically it is mostly visible as a longitudinal line on the body of fish. It consists of a series of separate small organs. These are also found on the head, viz. in a row above the eye, another under it and a third row along the gillcover and the lower jaw (see figure 10).

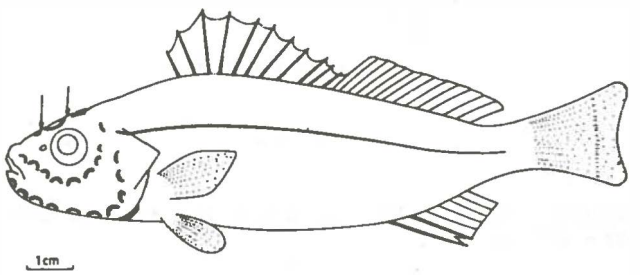


Figure 10. Acerina cernua (L). The heavy lines mark the lateral line organ; between the arcs on the head a cupula is found. The cupulae which are usually used in the experiments are indicated by arrows.

The organs proper are placed in furrows and often even in closed canals in the skull.

In several kinds of animals the topographical distribution on the body is rather variable and with regard to the development of the organ, variations have been found in one and the same species. In minnows originating from running waters the sense organs are placed in closed canals, which is

not the case with animals from stagnant waters. In salamanders there is a variation according to the seasons. Here the sense cells are found in follicles on the skin. When after the reproductive season these animals change over to a terrestial life, the follicles disappear under the horny part of the skin. In larvae of Anura and in some Amphibia, species constantly living in water, the lateral line organ is well developed.

It is noteworthy that in what we might call „bad swimmers among fish" the organ is usually ill-developed.

There are some modifications of the lateral line organ which, however, we can still recognize as such on the ground of morphological criteria (for an extensive review see Dijkgraaf 1952).

The lateral line organ develops from a placode in the centre of which lies the origin of the labyrinth in all vertebrates. The neuromasts of the canals on the head develop from the front part of the placode, from the back part those of the lateral line on the body (fig. 11).

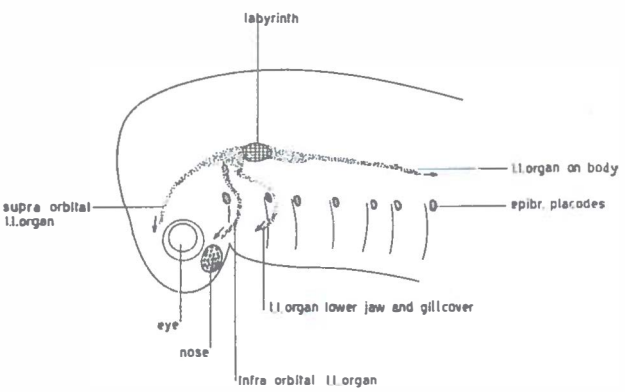


Figure 11. Development of the lateral line organ and the labyrinth organ from the same placode (According to Raven).

Consequently the lateral line organ is ontogenetically allied to the auditory organ and the equilibrium organ. Contrary to the structure, the innervation is much less variable. The system on the head is innervated by the nervus facialis, the parts situated behind the labyrinth by the glossopharingus and a branch of the vagus, known as nervus lateralis.

The lateral line organ proper consists of a group of sense cells on the top of which a gelatinous pillar is found, the cupula. Sense cells are more or less pear-shaped cells and most authors draw a sense hair on them. The length of this hair in the drawings varies from 0,2 - 5 times the length of the cell (see a. o. Dijkgraaf 1952).

Around this layer of sense cells elongated cells are situated, the supporting cells. These enclose the sense epithelium both from below and at the edge.

Only a few histological findings are mentioned in the literature on the structure of the cupula (Dijkgraaf 1934). De Vries c.s. noted that the cupula of the ruff had negative double refraction. This means that if light is propagated perpendicular to the long axis of the cupula, the component polarized in the direction of the striations is faster than the other component. The surrounding wall has a positive double refraction with respect to the striations there.

Katsuki, Y. c.s. observed a fibrillary structure in the cupula of the lateral line organ of the Japanese eel.

Some former investigators (Leydig, 1851) found striations in the cupula; these are often mistakenly called hairs.

Structure of the lateral line organ of Acerina cernua

Macroscopical examination

The canals on the head of Acerina are well developed and especially the cupulae of canalis supra orbitalis are extremely large: about 0,5 mm. Figure 10 gives a survey of this canal system. The dermatocranium forms furrows, locally covered by bony bridges under each of which a cupula is found. All this together is covered by the skin. The roof of the canal consists therefore of a number of fixed points between which the skin is stretched as a membrane. Through small openings in the skin the lumen communicates with the outside.

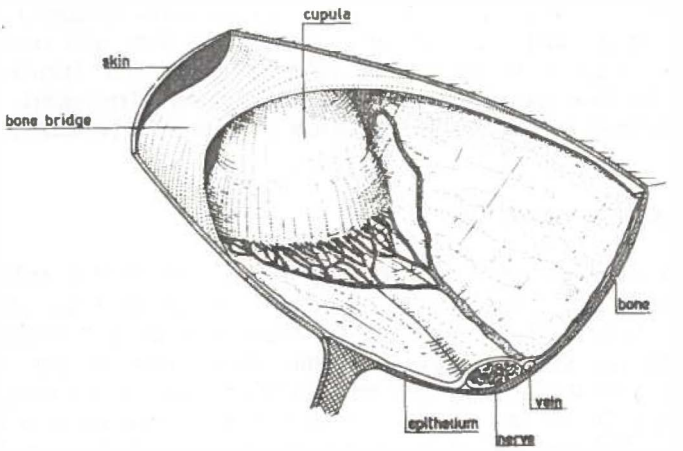


Figure 12. An single lateral line organ on the top of the head. The skin forming the roof of the canal is removed.

In figure 12 a semischematic picture of the second cupula of the canalis supra orbitalis is given.

After removal of the skin an orange spot can be seen under the bony bridge, surrounded by a paler ring. This spot is the sense epithelium, also called neuromast or macula cupulae. Towards the macula runs a white bundle of nerves. Parallel to the nerve run a number of veins, in which blood can be seen flowing from the cupula. Under strong magnification a mesh work of bloodvessels can be seen in the yellow macula.

Of the crystal-clear cupula nothing is to be seen as yet. It becomes visible by injecting a suspension of ZnO_2 or of aluminium particles into the canal. Figure 12 gives a picture of the appearance of the cupula. It has the orange-coloured spot of the macula as a basis. Around the cupula the wall can be seen. It is also transparant and reaches up to about half the height of the cupula. The wall is situated just above the paler ring of the macula.

For the rest no structure can be recognized, neither on the cupula nor on the wall. After detachment from the macula it is possible to see microscopic streaks in a not quite fresh cupula. Under the bony bridge there is a membrane connected with the bridge. The top of the cupula reaches up to this membrane. Two ledges run across it. Possibly these ledges determine the maximum amplitude of the cupula.

The cupula does not entirely block the canal. Laterally there is some space between the cupula and the epithelium on the inside of the canal.

After entire or partial removal of the cupula we have never observed regeneration. Usually the macula degenerates. Neither does the cupula recover its original state after the infliction of damage, e. g. removal of the top. I have, however, observed that the entirely free and undamaged cupula is capable of growth. Sometimes an organ that has been prepared free may rise in a few weeks above the upperside of the skull. In that case the top is usually bent forward. I have never observed any influence of this on the electrical activity.

Histological examination

Cupula. In microscopic slides little is left of the gelatinous cupula when the classic histological technique is used, in which the tissue is dehydrated in solutions of ascending percentages of alcohol. What remains then lies on the sense-epithelium like a pancake. It consists of fibrils running perpendicularly from the layer of the sense-epithelium (figure 13 and 14). On the borders of the cupula the fibres incline by a shrink effect. In some preparations coarser spiral fibres, stained dark by nuclear stains (Haematoxyline in this case),

are found. In a direction perpendicular to the sense epithelium the fibrillary structure varies in clearness in the slides. Towards the top of the cupula the fibres become clearer and clearer. In some slides there is a marked transition in clearness.

In none of the preparations could any connections between fibres and sense cells be demonstrated. There was always an empty space between the upperside of the macula and the lowerside of the cupula (figure 13). This may be an artefact and result from difference in shrinkage; it is also possible that in this place there has been a lipoid, which has dissolved during preparation. On account of its insulating property, which is of interest in connection with a model of de Vries (see chapter VI), special attention is paid to the existence of a lipoid layer. Some findings from frozen slides of a cupula fixed in formalin suggest that possibly there is some lipoid on the sense cells. With the polarisation microscope we occasionally found a double refractive layer on top of the sense cells. Sudan failed to show a lipoid in this place, however.

With the freeze-drying method nothing was obtained to indicate the presence of a lipoid on the sense cells.

I have gained the impression that there is indeed a special delicate structure between the cupula and the upperside of the sense cells, but that there is no lipoid in it.

The striation which is sometimes seen in the cupula may be caused by the fibrillary structure of the gelatinous cupula. These fibrils are the "hairs" described by older investigators. In the wall around the cupula we found a horizontal fibrillary structure in the slides prepared in the classic way. Here too the direction of the fibrils corresponds to that of the striations observed by de Vries c.s. 1952. The wall was connected with the macula in contrast to the cupula itself. The connection between cupula and the wall was not clearly visible in these slides.

Although in the slides the gelatinous structure has shrunk strongly, the findings support the observations of de Vries c.s. 1952 on fresh cupulae.

The macula. In the macula we find two kinds of cells; a layer of genuine sense-cells and around it elongated ones, referred to in literature as supporting cells (figure 15).

Round the basis of the sense cells a meshwork of nerve fibres can be demonstrated.

If the macula is prepared free from the connective tissue it behaves as a rather stiff disk. This is probably caused by the supporting cells.

Sense-cells. These are more or less pear-shaped cells, the narrow part of which is directed towards the lumen of the canal. The top of the narrow part adopts a bright red colour with Mallory-Azan.

The sense hair is clearly visible on each sense cell, especially in the slides fixed in Bouin fluid with pH 7,0 (figure 16). Its length is about 7 μ.

The nucleus is found in the lower part of these cells, where the cell plasm is far less compact than in the upper part. Mitochondria, small granules in the cytoplasm, are frequently found in the upper part, but are seldom present in the lower one. It appears from several recent publications that a large number of enzymes have been located in the mitochondria of animals cells. This may mean that in the case of the sense cells enzymatic processes are more frequent in the upper part of the cell than in the lower one.

Among these cells a few small cells are occasionally seen. I have obtained the impression that these are moving in the direction of the cupula. They may be compared with similar cells found by Vilstrup (1950, 1951) in the labyrinth of Acanthias. According to him such cells build op the gelatinous structure above the sense cells and the supporting cells; this has been demonstrated in the organ of Corti for the cells of Deiter (see below).

Supporting cells. Supporting cells are long (50 mμ), narrow cells, the proximal end of which forms a thin basement membrane between the lower side of the macula and the connective tissue under it. When there has been tension in a slide, e. g. caused by stretching the slides after cutting, a rupture is always seen at the basement membrane. Then the entire macula becomes detached from the connective tissue.

The place of the nucleus varies from cell to cell, but it is usually located in the lower part. The cells are rich in mitochondria.

Nerve fibres. Figure 17 gives a photograph of a cross section through a nerve to the second cupula of the canalis supra orbitalis. It contains thick and thin nerve fibres; both are myelinated. When a nerve fibre penetrated into the macula the myeline sheet disappears. On both sides of the nerve lie venous vessels. I have not made the time-devouring investigation of the ramification of these fibres. i. e. of the innervation of the cell. I have, however, studied the nature of the contact between nerve fibre and sense cell. There are two kinds of contact between nerve and sense cell.

In the one case the fibre ends in a knob on, or between the cells. In the other the fibre branches into a fine meshwork around the cell. Sometimes there is a basket of nerve fibres around the basal part of the sense cell. For the labyrinth organs the same kind of innervation has been described (e. g. Katsuki c. s., 1951).

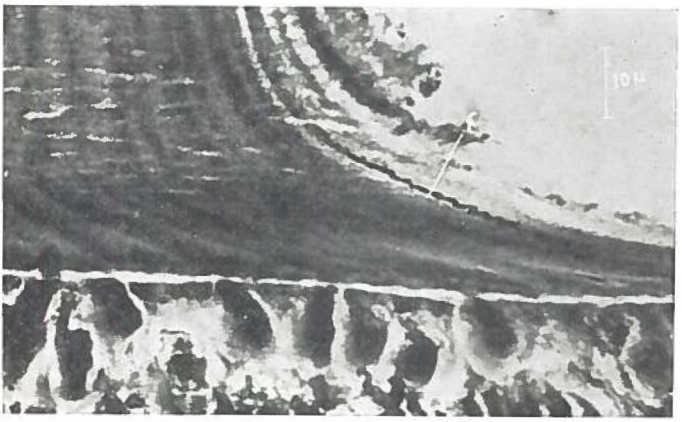
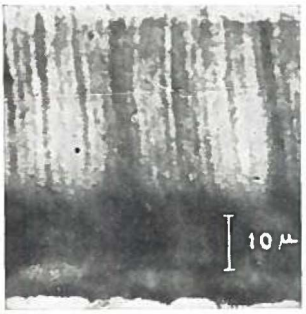


Figure 12.



finer
fibrillary structure
of the cupula

Figure 13.

PHOTOGRAPHS of histological preparates of the lateral line organ.

Figure 12. Bodian preparate of the cupula. Mark the darkstained fibre (f) and the space at the upperside of the sense cells.

Figure 13. Fibrillary structure of the cupula.

Figure 14. Longitudinal section through canal of lateral line organ. The cupula disappeared during preparation.

Figure 15. Sense epithelium with supporting and sense cells. Hairs on the sense cells.

Figure 16. Cross section through a nerve. 1 mm from the cupula. Thick and thin fibres.

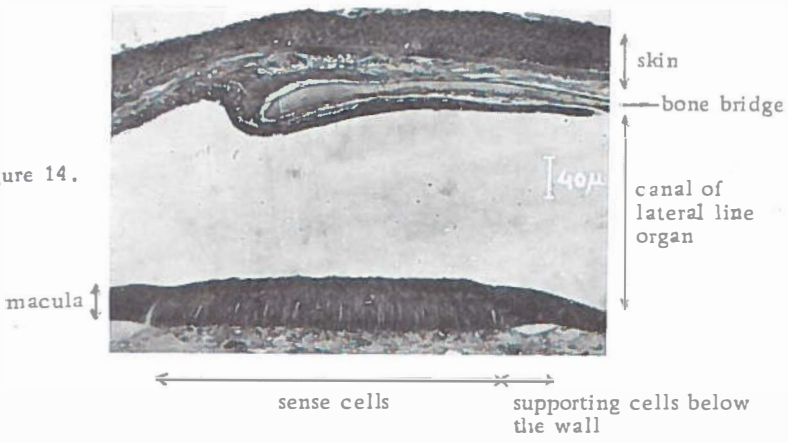


Figure 14.

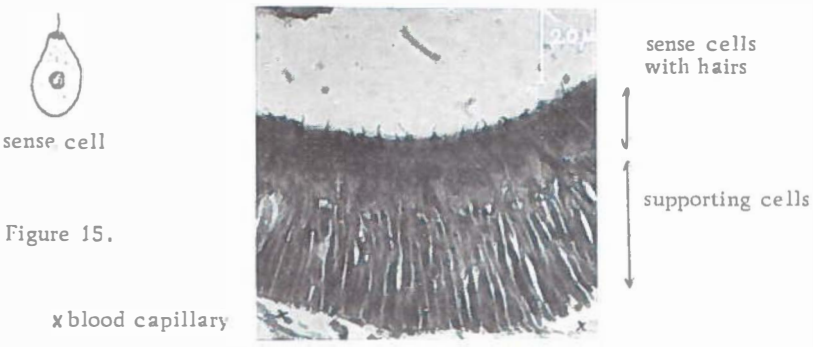


Figure 15.

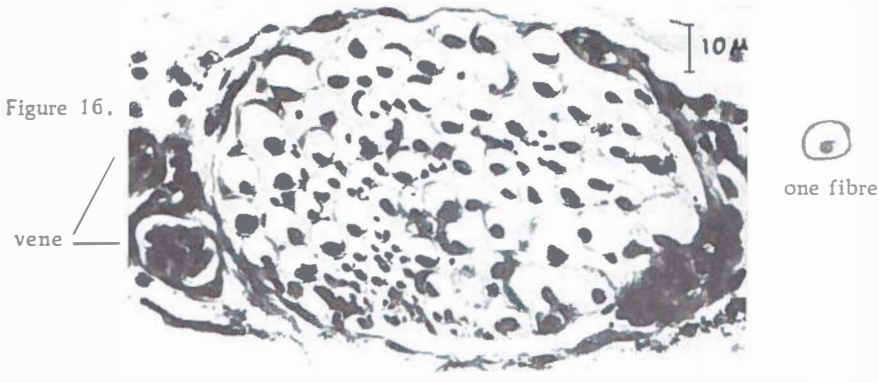


Figure 16.

cross section. nerve.

Blood supply.

Under the cupulae on the top of the head a branch of Arteria supra-orbitalis runs through the dorsal wall of the orbit to the maculae. It forms a meshwork of blood capillaries just under the basement membrane of the macula. The venous vessels together with the nerve run in the caudal direction. See figure 12 and figure 17.

Comparison with structures of the labyrinth

As already stated, the lateral line organ is ontogenetically closely related to the labyrinth. Examination of the microphonic effect proved that there is also a marked physiological resemblance. It is therefore of importance to examine in how far there is a structural similarity between the sense cells of these organs.

Many histological examinations of the labyrinth have been performed. I shall mainly confine myself to a discussion of the recent literature, with the emphasis on the sense cells.

The semicircular canals.

These organs show a marked structural resemblance to the lateral line organ.

After an extensive examination of the Acanthias, Vilstrup (1950) came to the conclusion that the cupula consists of fine fibrils running longitudinally in the organ and embedded in a fluid. The fibres originate in the sense epithelium.

Wersäll (1954) examined the sense epithelium of the semicircular canals in the labyrinths of caviae with an electron microscope. On the sense cells he found about 40 sense hairs, each about 10 μ long and 0,4 μ thick. The hairs consisted of an axial fibre surrounded by a tube formed by the cell membrane. In the photographs they are perpendicular to the cell membrane.

On the apical side the sense cells have a compact granular structure. Under the nucleus of the cells a number of membrane-like structures are found; they are of the kind described by Engström c.s. (1953) for the inner hair cells in the cochlea. These membranes were only observed in the flask-shaped and not in the longer cylindrical type found closer to the edge of the crista. The innervation of these types of cells differs. The flask-shaped cells were supplied by thicker fibres forming a basket of nerve fibres around the basement of the cells. The cylindrical cells, on the contrary, were innervated by the thinner fibres forming cup-shaped terminals on the cellular membrane.

It is noteworthy that, at the apical side of the supporting

cells, Wersäll found additional cellular terminals, apparently closely connected with the cupula. Vilstrup already found similar structures in the semicircular canals of Acanthias.

Both Vilstrup (1951) and Engström c. s. (1954) found similar structures for the macula utriculi in Acanthias and Caviae respectively.

For the cupulae of the semicircular canals of the Acanthias Vilstrup described a "subcupular zone" similar to the one I observed in the lateral line organ.

The Organ of Corti.

To us the connection between the sense cells and the tectorial membrane is of particular interest, It is, however, remarkable that in the histological literature this connection is poorly depicted. Often the tectorial membrane is drawn turned back from the organ of Corti; this is the usual situation in histological slides which have not been prepared very carefully. De Vries (1949) studied the structure of cochleae frozen in liquid oxygen; then the bone becomes very brittle and can easily be broken, whereas the various parts of the organ of Corti keep their original positions. He observed that the tectorial membrane was closely connected with the organ of Corti. His conclusion was that probably the hairs of the sense cells form the link. Examination in vivo e. g. by von Békésy, made it certain that in natural position it is closely connected with the sense cells.

In more functional pictures of the organ the tectorial membrane is drawn as an independent unit resting on the hair cells. It is well known that the tectorial membrane, a non-cellular structure, develops from the organ of Corti: consequently we should consider it a kind of secretion of the cells of the organ. Traumatic experiments with loud sounds prove that when disconnected from the organ of Corti, some degeneration appears. Engström and Wersäll (1953) found by means of the electron microscope that the supporting cells in this organ form protrusions which emerge in the direction of the tectorial membrane. Although these authors do not state this explicitly, it is highly probable that these extracellular protuberances produce the structural elements of the tectorial membrane.

I think we have here a situation similar to that in the other labyrinth organs and likewise in the lateral line organ. This seems to be much more probable than assuming a formation of the gelatinous structure by the sense cells. The tectorial membrane, too, has a fibrous structure (de Vries 1949, Katsuki and Cowell 1953). These fibrils originate in the protrusions of the supporting cells. In this way the connection

between the tectorial membrane and the organ of Corti may be built up. Moreover the meshwork on the tectorial membrane, rediscovered by de Vries, might have a mechanical function in this connection. On the sense cells of the cochlea of the guinea pig hairs are found on each cell, about 70 of approximately 4 μ length and arranged in a V-shape (Katsuki and Cowell 1953, Engström et al. 1953/1954). Here, too, the sense hairs have a quite different structure from the fibrils of ciliary epithelium: they are of the same type as the one described for the other labyrinth organs.

Conclusion

Structurally there is a marked resemblance between the sense cells of the various labyrinth organs as well as between these cells and those of the lateral line organ in fishes. In all these sense cells the cytoplasm of the apical part has a compact structure. On the sense cells a number of short hairs are found, whose number and length vary according to the various organs. Consequently morphological evidence for de Vries' hypothesis has been furnished. The thick nerve fibres form a meshwork around the cells. Thin fibres end in "boutons" on or between the sense cells. The gelatinous structure should probably be considered a product of the supporting cells and not of the sense cells. The fibres described by Vilstrup for the labyrinth of the Acanthias are not the sensory fibres, but the construction elements of the cupula.

In the lateral line organ the supporting cells form a solid frame in the form of a disk, in the centre of which the sense cells are situated and on which the cupula rests.

Any evidence of a "rigid" morphological connection of sensory hairs with the gelatinous part is lacking.

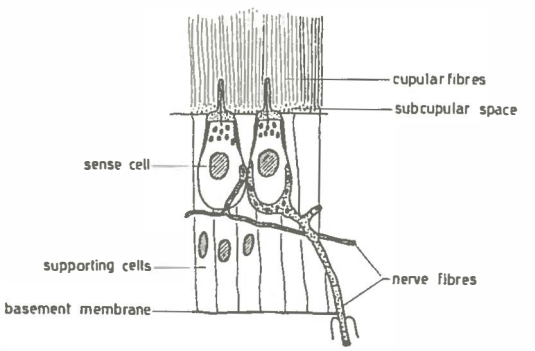


Figure 18. Sense epithelium of the labyrinth and the lateral line organ. In the sense cells the mitochondria are indicated.

Schematically the sense cells of labyrinth and lateral line organ may be represented as in fig. 18. A solid frame is formed by the supporting cells, which also build up the gelatinous structure. They form extracellular protrusions from which fibrillary elements arise. The short hairs on the sense cells are 4 - 7 μ long and are probably perpendicular to the cell membrane.

Chapter IV

A SURVEY OF LITERATURE ON THE MICROPHONIC EFFECT OF THE LABYRINTH ORGANS

This chapter contains a short survey of the literature on the microphonic effect with the purpose to inform the reader about the properties of the effect of the labyrinth organs. Subsequently it will be demonstrated that the microphonic effect of the lateral line organ has similar properties.

Introduction

In the surroundings of the auditory organ an electric activity can be measured which in closer investigation appears to originate from the cochlea. It is an alternating potential of the same frequency as the sound stimulating the organ, This potential is named by Adrian (1931) "the microphonic action of the cochlea". Other current terms for the same phenomenon are "microphonoc effect" and "Wever and Bray effect"; the latter after the discoverers.

The phenomenon has now been observed in all the Vertebrates that have been examined: human, monkey, dog, guinea pig, cat, rabbit, hamster, mouse, opossum, alligator, turtle, frog. It is not restricted to the cochlea only but is found in all the labyrinth organs (see de Vries and several coworkers, Zotterman and Pumphrey).

At first it was thought to be a nerve effect. Experimental investigations, however, have shown that this is not the case, but that it originates in the sense cells. Nerve effects may indeed be superimposed. In the guinea pig the nerves could be degenerated in a number of cases without decrease of the phenomenon, at least as long as the sense cells in the organ of Corti were intact (Neff 1947 and Wever and Neff 1947). When the sense cells are destroyed by certain pharmaca without impairing the nerve fibres, the effect disappears (Juul and Vraa-Jensen 1946 and Caussé 1951, Rüedi c.s. 1951 and 1952).

A biologically very neat technique is used by McCardy c.s. (1940), Larsell et al. (1935). The investigators examined the appearance of the microphonic effect during the development of a marsupial (Opossum). They could not find any microphonic effect as long as the hair cells and the supporting cells had not developed.

Recently von Békésy (1952) has studied the origin of the microphonic effect of the cochlea. He examined the sources proposed in the course of years by various investigators and came to the conclusion that it originated in the organ of Corti.

Of importance is the question of the energy relations. It has been supposed that the organ of Corti was a transformer which passively changed mechanical energy into electric energy. In that case the latter can never be greater than the former. Regarding the cochlea von Békésy (1952) has proved that the electric energy is greater than the mechanical energy, and de Vries (1952) came to the same conclusion for the lateral line organ. So here we are not dealing with a kind of piezo-electric property of these organs.

We may conceive the microphonic effect as an indication of a current around the sense cell. The size of this current depends on the strength of the stimulus on the cell. This current activates the nerve fibres around the cell.

Any direct proof of this conception has not yet been given. There are, however, indirect indications. The experiments by Davis c.s. (1950) show that, as a result of the operative procedures, the nerve effects (see below) disappear first, and only after that the microphonic cochlea effect, never the other way round. Kahana c.s. (1950) could block the nerve effects reversibly by lowering the body temperature of the hamster, without losing the microphonic effect of the sense cells. Tasaki (1953) found that afferent impulses in the acoustic nerve are initiated in the period during the increase of electric current which is generated by the hair cells and flows from scala vestibuli to scala tympani. His conclusion is that at low frequencies "the microphonics are the internal stimulus, which excites the afferent nerve-endings electrically".

Properties of the microphonic potentials of the labyrinth organs

The wave form of the effect

The wave form of the potential of the cochlea resembles that of the sound causing the effect. Careful investigation, however, reveals various dissimilarities depending both on the amplitudes and on the frequency of the sound. Firstly, at loud sounds harmonics become significant. Secondly, together with the microphonic effect a nerve effect is also recorded and this causes a deformation of the sinus form. At low frequencies (below 1000) the latter phenomenon becomes especially marked. This nerve effect is not, as is often thought, an action potential of the 8th nerve, but it is an effect of

ganglia. Of the nerve spikes themselves nothing is seen with the technique commonly used in these investigations. With the use of clicks two nerve effects can be demonstrated: they are called N_1, originating from the ganglion of Corti, and N_2 from the cochlear nucleus (Davis c.s. 1950, Kahana c.s. 1950).

Both the latent period and the amplitude of nerve effects vary with the temperature. The latent period of the microphonic effect is independent of the temperature and amounts to about 0,1 msec. Neither for the microphonic effect of the cochlea nor for that of the crista do we find any refractory period, nor anything of the all-or-none law known in the nerves (Kahana c.s. 1950).

With the use of a fine needle movable in various ways, von Békésy (1951) has shown that in the cochlea of the guinea pig the microphonic effect is only connected with the displacement, and not with the motion (velocity) of any part of the cochlea as such. Using a trapezium-formed wave, he obtained an output of this form. For the lateral line organ de Vries c.s. (1952) also came to the provisional conclusion that the potentials of the cupula were determined by the amplitude and not by the velocity of the cupula. He thought the same applied to the labyrinth organ (de Vries et al. 1952).

Von Békésy (1952) has observed that in the cross-section of the cochlear duct the direction for the most effective movement varies for different parts of the tectorial membrane

Influence of the frequency

It is well known in audiology that the sensibility of our auditory organ, i.e. the number of ergs required to get a sensation, is not the same at different frequencies. Usually the just perceptible energy is plotted against the frequency. This is a kind of equal response curve, which is called the audiogram.

For the microphonic effect a similar procedure is used. Then the energy necessary to obtain a constant voltage is plotted against the frequency.

In the audiogram the central nervous system is included, which is not the case with the microphonic effect. However, both curves agree fairly well, apart from a difference at the lower frequencies.

For the semicircular canals Huizinga, de Vries and Vrolijk (1951) have compared the frequency characteristic of the sensibility curve of the Tullio reaction (a specific reflectory movement of the head of a bird, with a small opening in a semi-circular canal, during administration of sound). Here too, a good resemblance was found.

Consequently the microphonic effect gives us a good idea about the sensibility of the entire sensorial system.

In beautiful experiments von Békésy has shown an increase of the elasticity of the basilar membrane, markedly along the cochlear tube. So the resonance frequency of the membrane varies. The same applies to the frequency sensitivity of the microphonic effect.

Influence of the amplitude

When the sound becomes louder, the microphonic effect increases proportional to the amplitude of the sound vibration. When a certain value is reached, it still increases, but no longer in proportion to the strength of the stimulus. At a certain maximum value further increase of the loudness diminishes the amplitude of the microphonic effect. After a strong stimulus a weak sound will then cause a smaller effect than before. This effect, hysteresis, is found in the cochlea of the guinea pig (Stevens and Davis) and in that of the pigeon (Bleeker). Some years ago, Vrolijk described a similar phenomenon for the crista effect. It is not certain that this is caused by a mechanical damage of the sense cells, for traumatic effects appear at stronger sounds.

Closely related to this is a phenomenon resembling adaptation. If we apply a constant tone of medium strength, the microphonic effect decreases during the first minutes; afterwards a constant amplitude is maintained, Ubbens (1955).

Blood supply

When the heart stops beating the microphonic effect quickly sinks to 1% of the original. This remaining potential originates in other membranes and has little to do with the "effect of the sense cells". These results make it evident that blood supply forms an indispensable factor for the cochlea effect. So the microphonic effect is a biological process which is closely connected with metabolic processes.

Anoxia and diminished oxygen supply cause a decrease of the microphonic effect (Lawrence and Wever 1952, Gisselsson 1954). The latter author could demonstrate that after cyanide application an increase in the amplitude always took place just after a respiration movement. The same phenomenon is described by Riesco, McClure, Davis, Gernandt and Cowell (1955).

The influence of chemicals

The influence of various pharmaca on the microphonic effect has been investigated. These investigations usually start-

ed from substances which in otology are known to have a specific poisonous effect on the labyrinth organ. The results of these investigations are rather varied. Concentrations that are clinically effective, do not seem to influence the microphonic effect. Probably these poisons do not affect the sense cells but the nervous system. Davis, Gernandt and McClure (1950), like van Eyck, succeeded in suppressing the nerve effect in the guinea pig and the pigeon respectively by injecting the animals with quinine. It is noteworthy that in the case of the pigeon Vrolijk did not succeed in this.

For our study those substances which influence the membrane potentials are of importance: e.g. sodium and potassium chloride. Tasaki and Fernandez (1952) could block the electric sense cell effect reversibly by isotonic solution of potassium chloride, brought into the cochlear duct. This is a clear indication that we are here dealing with a mechanism similar to that in the membrane potential of the nerve.

Curare is a substance which is known to influence the membrane potential. Although it is often used, also in doses fatal to the animal (van Eyck), no influence on the electric effect has been reported.

The influence of the temperature

If we are concerned with a series of different physiological processes, which are difficult to separate, it is often possible to unravel them by changing the temperature. One process will then be more strongly influenced than another.

The regulation of the temperature of mammals and birds makes an investigation of the influence of variation in the temperature in these animals difficult. In order to eliminate this factor Kahana, Rosenblith and Galambos (1950) used hamsters as experimental objects. At low temperature the animal hibernates and during this process its body-temperature decreases considerably. When this animal is narcotized, its temperature can be varied between 18° and 38° C. These authors found that within this range, the latent period of the microphonic effect — in a narrower sense — did not change. The amplitude of the nerve effects and of the sense effects was influenced by the temperature. The same was the case with the latent period of the nerve components.

Conclusion

The microphonic effect is an electric effect observed in the labyrinth organs. It consists of a primary effect, produced by the sense cells and a secondary nerve effect. The latter can not be recorded when the former is lacking, but the reverse is possible. In the cochlea it is produced by the

sliding movement of the tectorial membrane over the sensory epithelium; in this organ it depends on the relative displacement and not on the velocity.

The effect depends on metabolic processes.

Chapter V

MECHANICS OF THE RECEPTORS

In the morphological part it has been proved that in the sense organs of the labyrinth as well as in the lateral line of the fish we repeatedly have to do with a gelatinous structure, based on the sense cell epithelium. With this gelatinous structure the distal parts of the hairs of the sence cells are connected. (For this connection there is, however, no morphological evidence.) When e. g. the cupula slides over the sense cells, as suggested in the hair hypothesis, the hairs will be pulled out. This pulling of the hairs (twice each period) may cause an electric activity in the organ.

In this chapter I shall discuss the mechanics of the cupula both on the ground of former investigations by de Vries, Jielof and Spoor, and of own observations.

Moreover direct experimental evidence will be given for the sliding movement of the cupula in the natural situation.

1. Possible movements of the cupula

In the course of the years in which the labyrinth organs have been investigated, various suppositions have been made about the manner in which the sense cells in these organs are stimulated. It has been assumed that a change of the pressure on the epithelium, the pulling of the hair, deformation of the whole sense epithelium etc. produced by the movement of the gelatinous structure might be the adequate stimuli for the sensorium. The investigations of a. o. van Holst, de Vries and von Békésy have made it clear that the shearing movement of the gelatinous structure over the sense cells is the adequate stimulus to the sense cells.

To give the reader an idea of our arguments in favour of the shearing movement of the cupula, I shall discuss below the pros and cons on the basis of a number of models given in fig. 19. The upper block represents the cupula and the lower the sense epithelium in this figure.

1. One possibility would be that the upper block is lifted from the lower, so that the hairs are pulled (fig. 19-2). It is an old idea about the principle of action of the labyrinth organs. But the cupula is surrounded by the wall, which fits fairly closely to it. This means that such a movement would create a vacuum, which is impossible at the small forces we are dealing with here.

2. The upper part can be stretched (fig. 19-3). It may consist of stretchable protein molecules and as the sense hair is connected with these molecules, this will also grow longer.

Von Békésy (1953) has proved experimentally that in the cochlea movement of the tectorial membrane in this "vertical" direction is not accompanied by electric activity. I found that the same holds good for the electric activity of the cupula of the lateral line organ. No electric effect was obtained when a force was applied in the longitudinal direction of the cupula; this was done with the aid of a small magnet, connected firmly with the cupula, and moved magnetically in a direction perpendicular to the sense epithelium.

Von Holst (1950) has shown for the utriculus in fish that here, too, the movement perpendicular to the sense epithelium was ineffective.

Regarding the cupula of the semicircular canals this direction of movement is expected to be inadequate for the sense cells, as the top of the cupula lies against the wall of the ampulla. Of course the same applies to the cupulae of the lateral line organ, since they are situated in closed canals.

It is highly improbable that these organs should contain a mechanism effective in a direction perpendicular to the sense epithelium.

The possibilities of movement in the other direction are greater.

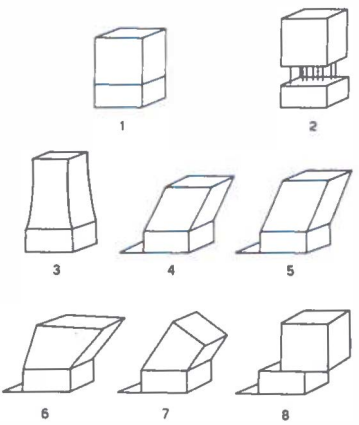


Figure 19. Models of the possible movements of the cupula.
1. The upperblock is meant to represent the cupula and the lower the sense epithelium; in 2 the cupula is moved up and down by a vertical force and this movement causes a stretching of the sense hairs; in 3 the cupula is stretched by a vertical force. When tangential force is applied the upper block might be deformed without a change in the length of the ribs so that the volume is decreased; in 5 only the rising ribs are lengthened, whereas the upper surface is extended, in model 6. In model 7 the upper block is bent whereas in 8 the whole block glides over the lower one. When a great tangential force is applied, there might be a displacement of both blocks, as indicated in figure 4-8.

3. In the case of a tangential force (model 4-8), the base of the upper block may remain in its place and when the length of the rising ribs is unchanged, only its form will after (fig. 19-4).

This model only shows a deformation (a "kink") at the base of the hair but no change in the length of the hair. In this case the volume decreases and such is impossible because of the small compressibility.

4. Fig. 19-5 shows a case, in which only the rising ribs change in length, so that the volume remains equal. For a vibrating cupula this means that the amplitude at the top is greatest and that the base is at rest. In this case the hairs are stretched a bit. An increase of the total length of the cupula could not be studied. The base of the cupula is not at rest, which does not correspond to the model. Though it does not seem the most probable one this model could not be excluded, entirely.
5. The upper surface might also become larger (fig. 19-6), while the rising sides and those of the ground square remain the same. I have never found indications for this in the lateral line organ. Vilstrup (1950) reports that a broadening of the top of the cupula can be observed in Dohlman's film of the movement of the cupula in the semicircular canals. Vilstrup did not explain this. When, however, the cupula bends and the basic surface as well as the length of the rising sides remains unchanged, a broadening of the upper side is the only possibility to keep the volume constant.

Because in this model there are no forces acting on the sense cells, it is not so very important to us at the moment. Moreover, removal of half of the cupula does not influence the microphonic effect.
6. Model 7 in fig. 19, a bending of the cupula is the most probable one at first sight. In this case the pressure side is stretched and the other compressed. This model was the starting point for de Vries' piezo-electric theory (1948). The idea was rejected since the greater part of the cupula can be removed without a corresponding decrease in the amplitude of the microphonic effect.

Yet the bending occurs as described by de Vries c.s. (1952) and frequently observed by me on the cupula if it has been prepared free. The bending may cause a stretching of the hairs, though in a slight degree. The double frequency of the microphonic effect can be explained when we assume that the hairs on the one side of the macula are stretched during half of the period and the others during the other half. In that case we expect the wave-form to alter when the electrode is displaced from before to behind the cupula. Then in the former case it is closer to the source of one peak of the microphonic effect (see fig. 1), whereas in the other situation the electrode is closer to the source of the other peak.

De Vries c.s. (1952) already found that the wave-form remains the same for all positions of the electrode. This was even the case when the electrode was brought into the cupula close to the epithelium, providing that the electrode did not displace the cupula.

So the bending of the cupula is not very likely to be essential for the microphonic effect. Moreover, when the bone bridge was still above the cupula, I could not observe any bending at all.

Dijkgraaf (1934) only described a bending of the long and free cupula of the pope (Phoxinus laevis). I am not sure that in this case the bending results in a stimulating action to the sense cells.

7. The last possibility is that the cupula as a whole is displaced, i.e. model 8. In this model, the displacement of the top equals that of the base. I have observed this in partly free cupula (in which only part of the skin of the canal is removed). This is a strong argument in favour of this model. In free cupulae the amplitude at the top is usually twice or three times as large as at the base (de Vries c. s. 1952). So there is a bending and a displacement of the whole cupula.

Regarding the hair hypothesis it is only essential that there is a shearing movement of the cupula over the sense epithelium.

From the investigations by von Békésy (1953), Huggins (1953) and Vilstrup (1950) it appears that this kind of displacement (model 8) is also the most likely one for the labyrinth organs.

2. Some new observations on the movement of the different parts of the cupula

It is of importance to go more deeply into the matter of the movement of the different parts of the cupula over the macula. Fig. 20-1 shows a schematic drawing of the situation. The wall should be considered a supporting element of the central part. The results of the morphologic investigation suggests a close connection between the wall and the macula. After careful partial removal, we can pick up a normal microphonic effect. So it does not seem to have a function in the production of the microphonic effect. In shifting of the central cupula the wall forms an obstacle.

We have more or less the following situation: in the centre of a dish formed by supporting and sense cells, lies a gelatinous pudding on a layer of fluid. Around this central pudding lies a gelatinous ring, firmly connected with the dish. The inner side of the ring is connected with the central pudding.

I have tried to find out what happens at the base of the wall. At large displacements (20 μ and more) of the cupula (only skin caudate of the bone bridge removed) caused by water vibrations, it was clearly visible how the wall bent over small ZnO_2 particles at the foot of the wall. The particles (diameter approximately 10-25 μ) disappeared for a moment but later on became vaguely visible through the wall (fig. 20-2). It seems to me less probable that the wall slided over the macula because in that case the particle would be swept away.

It should be remarked that this could only be observed when the cupula had been used for some hours and had apparently

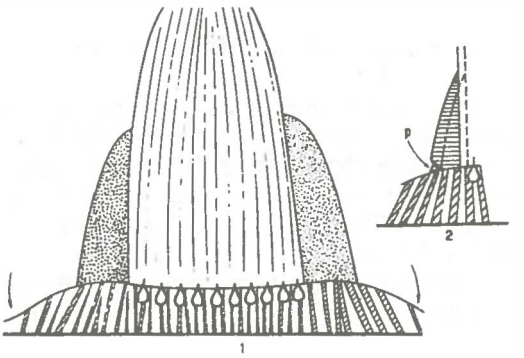


Figure 20. Schematic picture of the lateral line organ. The wall, the sensory cells with hairs, the supporting cells and the basement membrane are indicated. At great forces a displacement is seen at the places marked by the arrows.
In figure 2 p is a particle over which the wall bends, in cases of great displacements of the cupula.

become soft. Just after the operation of the cupula the effect could not be seen as the displacements were too small.

At least for the soft cupula a movement as indicated in fig. 20-2 is the most probable.

The macula is a rather rigid structure and when its connection with the surrounding tissue is weak, it is possible to see a displacement of the macula if great forces act on the cupula. I did sometimes observe movements of the tissue at the foot of the wall (the arrow in fig-20-1 indicates the place), but only at a cupula prepared free. It will be clear that these movements are unimportant for the excitation of the sense cells.

3. Special mechanical properties

For the animal it is on the one hand of importance to have an organ which is as sensitive as possible. The theoretical limit is established by the Brownian movement. On the other hand it must be capable of sustaining large displacements which will occur occasionally. It is clear that a very delicate mechanical system will be very easily destroyed. It is noteworthy how nature has solved this problem.

A study of the animal's sensibility to vibrations by training them shows that they are able to react to movements of the fluid in the canal of at least about 5 millimicrons (see chapter XII). During experiments on the microphonic effect it appeared that the organ could endure displacements of 50 μ without any noticeable effect on the electric activity.

A demonstration of a substance with small elasticity and great plasticity gave me the idea that such properties might also be found in the cupula. A little ball of the material mentioned bounces perfectly, but if a constant force is applied to

it, it behaves like putty. An example of such a substance is asphalt.

When we ascribe these qualities to the cupula we can explain some otherwise rather incomprehensible observations.

1. When a free cupula is drawn out of its equilibrium position it may sometimes take several seconds after its release before it is back again. This has already been described, but not explained by de Vries c.s. We consider it a symptom of the more plastic properties of the organ. The elastic forces which bring the cupula back to the equilibrium position are small.

2. I observed a similar phenomenon when the cupula was drawn by a constant force, e.g. a watercurrent. It suffers immediately a displacement which gradually increases. When the force is doubled, the immediate displacement is likewise doubled, and then the cupula slowly creeps further from the equilibrium position. This can also be seen in fish no. 5 of the publication of de Vries c.s. (1952). The displacement of this cupula under constant forces is large, compared with the experimental results of other fish, whereas the amplitude for vibration is not essentially different. Consequently the plasticity is increased, and the elasticity is about the same as in the other cases.

3. When the cupula is elastic as well as plastic, it will behave as an ideal elastic bar towards vibrations. When the organ is displaced out of the equilibrium position by a grain of sand or something, its sensibility to an oscillating force will not alter. Indeed we found, that the amplitude remained constant over a longer distance, see table 1.

Table 1

c	2a	c	2a
0	3-4	0	4
2	3	19+8	4
8	2-3	0	7
14	2-3	19+8	4-5

a = amplitude at 40 c.p.s. during a constant displacement c of the cupula. Both a and c expressed in μ. +8 indicates that during one minute the organ creeps 8 μ further.

These three points suggest that the cupula is indeed elastic to vibrations as well as plastic to constant forces. Plastic is here used as the opposite of elastic. Of course, when a constant force is released, elastic forces of the organ will bring it back to the equilibrium position, but apparently these are small.

These mechanical properties of the cupula have consequences for the connection of the sensory hairs with this organ. These will be discussed in chapter V (slip effect).

At the tectorial membrane von Békésy observed similar elastic and plastic properties. This membrane is soft for static deformations but rigid for vibrations.

It is interesting to see whether a constant displacement of the cupula influences the sense cells (see chapter VII-3).

For the determination of the stiffness of the cupula Jielof and Spoor (de Vries c.s. 1952) used constant displacements of the cupula of about 100 μ. They found a stiffness of 250-1000 dyne/cm. It is clear that in these measurements the mechanical properties of the organ mentioned above have been of importance. De Vries expected smaller stiffness for vibrations and used in his estimation of the resonance frequency f = 125 dyne/cm.

4. Observations on the oscillating cupula

It is instructive to consider the cupula as a harmonic oscillator. In the experiments we are discussing here the magnetic driving method was used. In case the magnet was placed on the cupula it was securely fixed to the organ to ensure good coupling. When the magnet was mounted on the skin the coupling is good because the water, moved by the skin, will practically not move along the cupula.

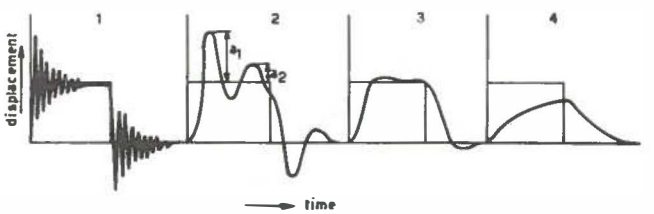


Figure 21. Movements of the oscillators with different damping factors. The force is indicated by the rectangle and the displacement of the oscillator by the curve. In 1 there is a small damping, in 2 the damping is considerable, in 3 just critical and in 4 it is more than critical.

In order to get an idea of what we can expect at the cupula we shall first consider a simple physical oscillator. A bar or a spring vibrates in a special frequency when it is moved out of its equilibrium position and released. This frequency is called the natural or the proper frequency, ν_o, of the oscillator. The amplitude vibrations gradually decreases; it is "damped" out. For our discussion ν_o and the "damping factor" are of interest. ν_o is determined by the elasticity (f) and the mass (m) of the vibrator.

$$\nu_o = \frac{1}{2}\pi \quad \frac{f}{m}$$

. Damping is determined by the "friction" (r).

When a_1 and a_2 are the subsequent displacements in the same direction (fig.21-2), of the damped vibrations, $a_1/a_2 = k$ is a criterion for the damping. Mostly used is $\Lambda = \ln k$, logarithmic decrement. In forced oscillations the frequency of the driving force, ν_u, will be usually different from ν_o.
In the case the oscillator is pushed the halftime value of the decrease of the amplitude of the vibration is $I = \frac{0.7}{\Lambda \nu_o}$

In fig.22 the relation between the amplitude (ordinate) of the driven system and the frequency of the driving system (absis) is plotted. The amplitude of the driving force was kept constant and assumed to be one. To produce a figure showing the whole frequency range ν_u/ν_o is used on the left hand half of the absis and on the right hand half ν_o/ν_u. The lower figure gives the difference in phase between the motion and the force. When ν_u is much below ν_o and moreover the damping slight, the amplitude is small and in phase with the force. Near the resonance frequency there is a jump in the curve; if ν_u equals ν_o (the condition of resonance) the displacement is 90^o in arrear of the force, so in phase with the velocity. Further increase of the frequency gives a displacement more and more opposed to the force.

The figures show how the phase relations and the resonance curves change at different damping factors.

In case we want a great amplitude at a special frequency, we must have a system like that presented by curve 1.

A detector of this kind is frequency sensitive. Even a small change in the frequency of the driving system causes a considerable change in the amplitude of this resonator.

This is not something we should expect in the cupula, for then the animal would only be able to perceive vibrations in a narrow frequency band. It is obvious that a more favourable situation for a vibration detector is represented by curve 4. Here a change in ν over a wide range causes no change in the amplitude of the driven oscillator and we need not be afraid of mechanical damage of the "suspending system" - in our case the sense cells - in the resonance frequency. This means that the mechanical part, i.e. the cupula itself will not work as a frequency analyser. This must be done farther on by the sense cells or the nerve fibres.

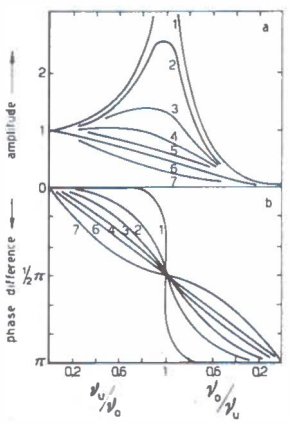


Figure 22. Resonance figure of a harmonic oscillator. ν_o resonance frequency and ν_u applied frequency; the damping increases with the numbers near the curves; 6: the case of critical damping.
A: relation between the amplitude and the frequency applied.
B: interrelation of the frequency and the phase difference between the force and the displacement.

Another question important for a vibration detecting mechanism is, that it must immediately indicate a change of the force and the frequency. A critically damped oscillator indicates changes of the driving system as quickly as possible. In this situation there is no maximum in the resonance curve (fig.22-6), neither is there a jump in the phase relation. For this type of resonator around the resonance frequency the product a. ν is constant over a wide frequency range.

If the damping is still further increased, the oscillator needs more time to perceive a change of the driving force: it creeps (fig.21-4).

The mechanism of the vibrating cupula has been described by de Vries c.s. (1952). They pointed out that the constants, mass, stiffness and friction cannot be measured very exactly. I have not tried to improve their experiments, therefore. I did measure the amplitude of the cupula at larger spectrum than de Vries c.s. did and in addition I have tried to get some idea of the damping factor indirectly.

Mechanical measurements on the oscillating cupula

During the experiments the amplitude of the cupula in a natural situation is so small that it cannot be measured. I only succeeded in exceptional cases. Fig. 23, fish 87, presents such a case. From October 30 onwards this fish was used daily during 2 à 3 hours for phase measurements on the microphonic effect. All these measurements gave the same picture. Also the measurements of November 4 do not essentially differ from those of the preceding days. On this day the fish lay on its side in the aquarium and its respiration movements were very irregular. Now the displacement of the cupula was sufficient for amplitude and phase measurements. The bad condition of the fish had apparently influenced the stiffness of the cupula. The measurements show that in this case the cupula behaves as a simple pendulum. Here the amplitudes at the top and the base were the same.

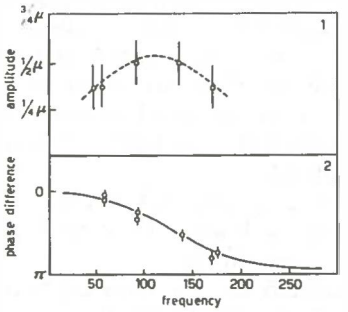


Figure 23. Mechanical frequency characteristic of a cupula when still under the bone bridge, fish 87. In 1 the amplitude of the cupula at different frequencies (cycl/sec) but the same amplitude of the force. In 2 the difference in phase between the force (topvalue of the voltage on the driving coil) and the maximum displacement of the cupula. The vertical lines indicate the error in the measurements.

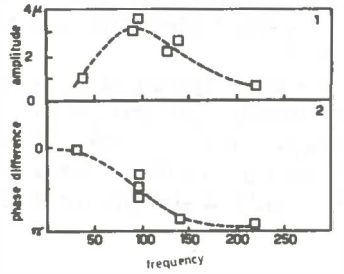


Figure 24. Similar to figure 23; in this case the cupula supplied with a magnet; Fish 95.

Fig. 24 gives similar measurements for a free cupula provided with a small magnet and driven magnetically. In this case the cupula was rather stiff, and the amplitude small. Here, too, the amplitudes at the top and the base of the wall were equal.

In both cases, as well as in several other measurements not published here, the resonance frequency agrees fairly well with the value expected by de Vries (1952): 80 c.p.s. At low frequencies the displacement of the cupula is in phase with the force. In the region of the resonance frequency there is a difference of 90°. Above this frequency the cupula is almost in opposite phase. The course of the phase curve indicates a considerable damping. This damping could be studied in more detail indirectly, viz. by pushing the cupula and at the same time measuring the electric activity.

If the cupula vibrates, this must result in an electric activity. Fig. 25 gives oscillograms made in such an experiment.

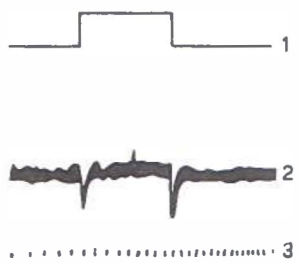


Figure 25. The electric response 2 of cupula when still under the bone bridge, when a magnet mounted on the skin is pulled; recording with a dc-oscilloscope. Curve 1 indicates the current through the driving coil, so this corresponds with the displacement of the magnet. Curve 3: time marks 1/15 sec.

The cupula was pushed by pushing a small magnet, mounted on the skin of the canal. The cupula could creep back, as the canal was not entirely closed and the fluid could move along the cupula. The oscillograms clearly show that there is only one oscillation and this means that in a natural situation there is probably critical damping of the lateral line system (cupula, the fluid in the canal and the skin).

In a magnetically driven free cupula this was often not the case (fig. 35). This was the only difference I was able to find between the results of these two driving methods.

For the sake of completeness it should be mentioned that the load of the magnet on the cupula had no effect on the amplitude and the phase of the microphonic effect when the cupula was driven by an alternating watercurrent.

In agreement with de Vries c.s. (1952), I found that at a given frequency the amplitude of the cupula increases proportionally to the force acting on the organ (see fig. 26). This was observed for all the frequencies at which I could measure the amplitude. Only at great displacements a deviation was found sometimes.

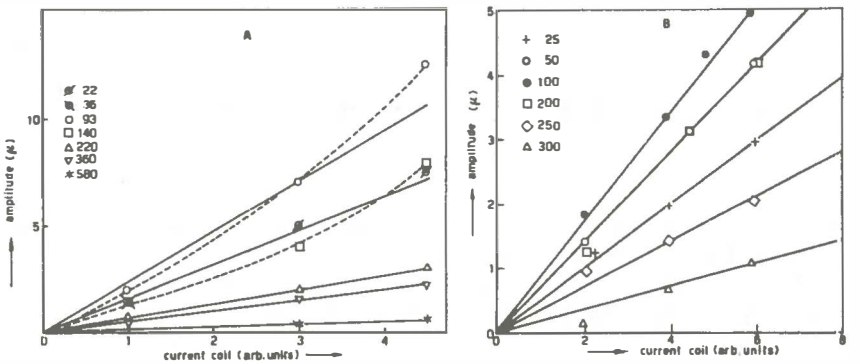


Figure 26. Amplitude of a cupula at different currents through the driving coil. The values near the signs indicate the frequency. In the case of fig. A at 93 and 140 cps. the relation between the amplitude of the force and of the cupula was not linear.

5. Conclusions

The most important conclusions of this part are:
1. In the natural situation, i.e. with the cupula still under the bone bridge, there is no bending of the cupula, but displacement of the whole organ. It is now known that similar shearing movements are adequate in the labyrinth organs.
2. To the cupula must be ascribed elastic as well as plastic properties. For static forces the organ is soft, whereas it is stiff for oscillating forces.
3. The behaviour of the vibrating cupula was studied between 10 and 500 c.p.s. whereas formerly this could be done up to 50 c.p.s. I found that the organ behaves as a simple harmonic oscillator, which is at least in the natural situation approximately critically damped. In this situation the amplitude is found to be smaller than one micron.

Chapter VI

OUTLINE OF THE HAIR HYPOTHESIS

In this chapter I shall describe the situation as it was when I started the electrophysiologic experiments. Next I shall give the more theoretical considerations on which the experiments, described in the following sections, are based.

As in the case of the labyrinth organs we found in the lateral line organ a jelly-like structure, here called the cupula, resting on a layer of sense cells (see fig. 20). When this organ is stimulated by watercurrents, electrical voltages can be recorded from the vicinity of the organ (de Vries 1948). This electrical effect is called the microphonic effect. De Vries, Jielof and Spoor (1952) have studied these voltages in more detail. The greater part of the experiments described in the next chapter are based on this study.

The most striking result of their investigation was that the frequency of the microphonic effect was twice the frequency of the movement of the cupula, ranging from 14 to 60 c.p.s. They found that the voltages above and below the organ were of the opposite sign, whereas the wave form did not depend on the position of the electrode. Between 14 and 48 c.p.s. the voltage only depended on the amplitude of the cupula; it seemed to be independent of the frequency. The measurements indicated that the double wave-form should be interpreted as two negative peaks; one corresponding to forward, the other to tailward displacement of the cupula. This was supported by the results of experiments in which the cupula was given a constant displacement superimposed on the vibration. When the constant displacement in, for instance, a forward direction was so large that the cupula could not pass the equilibrium position, one of the negative peaks (the one corresponding tailward phase of the vibration) disappeared. The other peak disappeared when the cupula was displaced in the other direction.

Observations of the movement of the cupula indicated that there was a bending as well as a displacement of the whole organ. In chapter V we have seen that in the more natural situation a displacement of the whole organ is observed only.

In order to account for these experimental results de Vries (1952, see also 1956) proposed the model given in fig. 27.

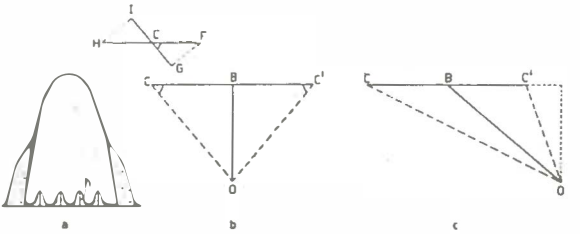


Figure 27. Schematic diagram of the lateral line organ with hairs h, b and c geometry of the movement of the hairs. BC and CF amplitude of a low and a high vibration respectively. The stretching component, IG, of the superimposed vibration increases with the increasing of CB.

On the sense cells there are hairs and these are assumed to be embedded in little holes in the cupula. The end of each hair is supposed to be connected with the cupula. If the vibrating cupula glides over the sense epithelium, the top of a hair is displaced from C to C'. As OC is longer than OB, this means that the hairs are pulled twice each period.

The idea was that *the voltage of the microphonic effect is related to the force acting on the hair.*

At this point I think it right to emphasize that it was never the intention to suggest that the voltage was produced by the hairs themselves, although I grant the terminology was sometimes misleading. From the outset in 1951, the conception was that the voltage was produced by the sense cells and that through the hairs the cupula acts on these cells.

As far as the connection between the cupula and the sensory hair is concerned the model of fig. 27 was a purely physical one, a mere geometrical speculation. A more realistic model concerning this connection may be found in an article by de Vries on "the Brownian motion and transmission of energy on the cochlea" (1952 J.A.S.A. page 530). The hair will be embedded in the gelatinous substance of the cupula and this substance is usually firmly attached to the hair. When the cupula is displaced the gelatinous structure is deformed because the elasticity of this substance exceeds that of the hair, so the "gelatine" will be stretched until its elastic force equals the force applied. The extent to which the gelatine is deformed is directly proportional to this force ($k = f.x$). The factor f depends on the dimensions of the hair and on the elastic constants of the material of the cupula (see, for a quantitative discussion, the literature mentioned above). This force is transmitted to the top of the cell by the hair.

For the calculations done in the following section it is immaterial whether we ascribe the elastic properties to the hair or to the substance in which it is embedded. To simplify the wording I will use the word hair. When in the following

parts expressions like "stretching of the hairs" are used, these will only have a theoretical meaning. It means, strictly speaking, that the gelatine is deformed and that strain is transmitted to the top of the cell by the hair.

With the assumption that the microphonic effect is related to the pulling of the hair, the doubling of the frequency is explained, since the hairs are pulled twice each period. Moreover, the model gives a simple explanation of what happens if the cupula vibrates while being displaced of the equilibrium position. In that situation, fig. 27-c, the hair is stretched only once each period, presuming that the constant displacement is larger than the amplitude of the vibration. In this case, it means that the double frequency will change into the single one. This was indeed found in the experiments.

In the ear no frequency doubling occurs. Here the sense cells are in an inclined position in respect to the direction of the movement of the gelatinous structure; when the same is the case with the hairs, the situation corresponds to that of fig. 27-c.

The third consequence of the model is the effect we get when a high frequency vibration with a small amplitude is superimposed on another with a lower frequency and a large amplitude. The former has a weak stretching effect on the hair. When the hair is in an inclined position, as a consequence of the large amplitude of the lower vibration, the small displacement of the cupula by the high vibration had considerably more effect (fig. 27-b). In terms of the electric effect this means that the microphonic effect caused by the high frequency is amplified by the lower one during the phase of the vibration period in which the cupula is out of its equilibrium position. At the moment that the cupula passes the equilibrium position there should be no amplification. Besides, the frequency of the electric effect, caused by the superimposed vibration, must be single during the amplification phase.

Unlike the other points, which were used to build up the hypothesis, the third one was checked afterwards. What happened in the experiments is demonstrated in fig. 28.

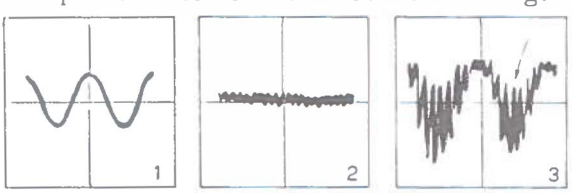


Figure 28. Microphonic effects of the lateral line organ.
Curve 1: voltage of the low frequency vibration.
Curve 2: voltage caused by the higher frequency with a small amplitude.
Curve 3: voltage curve obtained when both stimuli are applied; in the negative peak, the response of the higher frequency is amplified.

In the negative peaks of the lower vibration the effect of the higher one is single and its amplitude is considerably larger than when given alone. We see that there is an amplification as was predicted by the theory. This superposition effect seemed to be a good support for the hair hypothesis.

The next question we have to discuss is: *how are the sense cells able to produce the microphonic potential?*

In relation to this fundamental question de Vries (1956) has proposed the following model. It can be best explained on the basis of fig. 29.

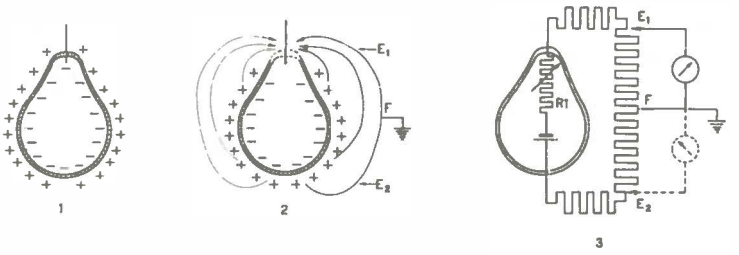


Figure 29. Model of the sense cell; 1 rest situation of the cell; 2 the currents are indicated (schematically) when the resistance of the cell membrane at the top of the cell is decreased. 3 the electric analogue: a battery and a changeable resistance R_1 represent the cell.

Across the cell membrane of most living cells – and we have indications that the same holds good for the cells of the lateral line organ – a potential difference is found; the inside of the cell is negatively charged as regards the outside, about 100 mV. This polarisation is related to a difference in the concentration of ions at either side of the membrane. When on any part of the cell membrane a "leak" is produced there will be a current into the cell as indicated in fig. 29-2. Now it is reasonable to assume that the upper part of the sense cells is predestined to become leaky as a consequence of the pulling on the hair. The extent to which the resistance of this part of the cell decreases will depend on the extent to which the hair is pulled. When the cupula oscillates, the resistance will fluctuate and consequently the current around the cell will alternate.

This current around the cell is supposed to stimulate the nerve fibres surrounding the cell.

The e. m. f. of polarisation is assumed to be produced by a pump moving ions from one side of the membrane to the other; this pump is driven by metabolic energy of the cell. Such a kind of pump mechanism has already been introduced in nerve physiology, see chapter IV. The electric analogue of the model is given in fig. 29-3.

In the sensory organ, in which we are not concerned with one, but with several cells, we measure the current of the cells as a potential difference across the resistance of the medium between the two electrodes; this is called the microphonic effect. The sign of the voltage will be reversed when electrode E is brought from 1 to 2 (fig. 29-2). Such a reversal was observed by de Vries c.s. (1952) when the pickup-electrode was replaced from above the organ into a position under the sense epithelium.

The hair hypothesis could give an unforced explanation of the quantitative experimental results obtained in 1952. Moreover, the superposition effect was discovered as a consequence of the model.

However, a closer inspection of the experimental results, especially as regards the quantitative measurements, showed some complications. De Vries, Jielof and Spoor (1952) already mentioned the following facts.

1. The constant deviation necessary to suppress one of the negative peaks was larger than the amplitude of the vibration.
2. A phase shift between the electrical output and the mechanical motion was noticed.
3. According to the model, the output for small amplitudes should reach zero sooner than the first power of the amplitude of the cupula, whereas the experiments gave a linear relation.
4. Sometimes the output curve was not a true sinus but had flat tops.

The first, the second and fourth point can be explained when a slip of the hair at its connection with the cupula is assumed. Then the hair will reach its moment of maximum tension before the moment of the maximum displacement of the cupula. Hence the electrical response will be maximum before the mechanical; this was indeed observed at low frequencies.

Moreover, the same will occur, when a constant displacement is given to the cupula. Then the hair gradually slips back and thus it becomes comprehensible that in order to change the double frequency into the single one, the constant displacement must be more than the amplitude of the vibration.

When we assume that the hairs are not rigidly connected with the cupula at small displacements (the hairs are "slack") and that they become so at larger displacements, then the flat positive tops can also be explained. In such a case there is no force acting on the sense cells and hence no microphonic effect when the cupula moves towards its equilibrium position.

Difficult to explain was the linear input-output curve and

the non-linear superposition effect. The former could be explained by assuming two groups of hairs, both inclined, but in different directions. One group is stretched when the cupula is moved in, for instance, a forward direction, the other when the cupula moves tailwards from the equilibrium position. But then another explanation must be sought for the superposition effect.

Outline of the experiments described in the next chapter

This was broadly the state of affairs when I started in 1952 with the experiments described in this thesis.

The first aim was a critical study of the difficults mentioned above. Therefore the hair hypothesis was worked out quantitatively, and as far as possible the theoretical consequences were checked experimentally.

As a first approximation it seems reasonable to assume that the processes induced in the sense cells by the pulling on the hair, i.e. the change in the resistance at the top of the cell, is directly proportional to the tension in the hair. According to the terminology described in the preceding section, this means that the voltage of the microphonic effect is in direct proportion to the extent to which the hair is stretched.

Or $$V = k.x \qquad (1)$$

where V is the voltage of the microphonic effect measured from top to top, x is the increase of the length of the hair and k is a constant.

On this assumption a number of calculations were made. To simplify the explanation of these the following symbols are introduced. See fig. 30-A.

B basic frequency or vibration on which a higher one is superimposed.

S superimposed frequency or vibration, usually with a small amplitude.

V_B voltage caused by the basic vibration

V_S voltage caused by the S-vibration.

W_S voltage caused by the S-vibration in the negative peak of the B-vibration.

A amplification factor, equals W/V.

I amount with which V_B increases by the S-vibration.

D amount with which V_B decreases by the S-vibration.

In the calculations on the hair model the following symbols are used: see fig. 30-1-4.

x extent to which the hair is stretched.

a_B amplitude of the cupula during the B-vibration.

a_S amplitude of the cupula during the S-vibration.

p extent to which x is increased by the S-vibration.
q extent to which x is decreased by the S-vibration.
u equals $p + q$.
e calculated amplification factor, u/x_S.

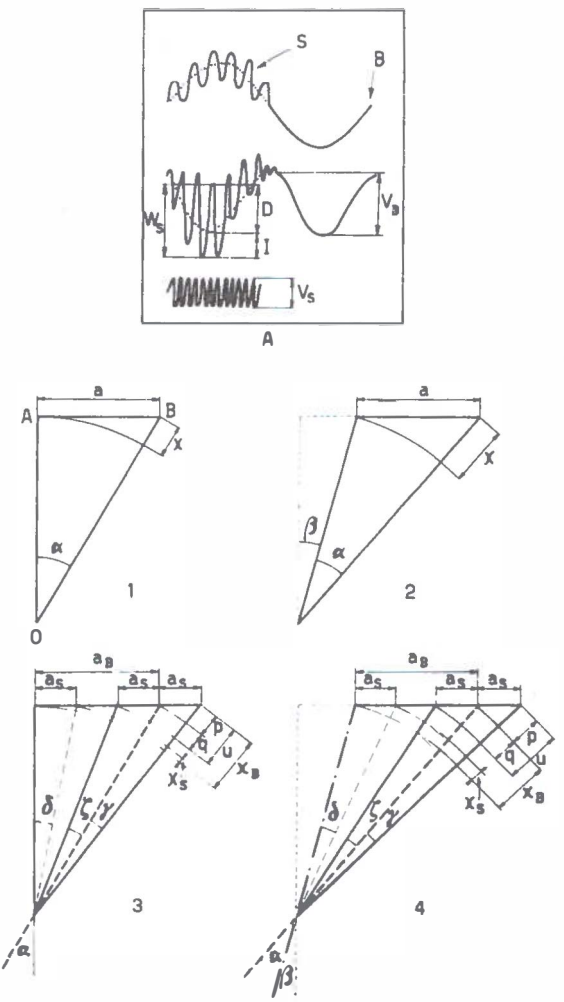


Figure 30. A: the symbols used in the text. The upper curve represents the movement of the cupula, the other the voltage curve of the microphonic effect. Figures 1 - 4: the extent to which the hair is stretched by the displacements of the cupula. In 2 and 4, β indicates the inclination of the hair when the cupula is in the equilibrium position. Figure 3 and 4 give the stretching effects of the amplitude of the superimposed vibration at the moment of the maximum displacement by the basic vibration.

For the voltage of the microphonic effect of the oscillating cupula can be calculated:

$$V = k\{(1 + a^2 \sin 2\pi\nu)^{\frac{1}{2}} - 1\} \qquad (2)$$

V: voltage of the microphonic effect; k: a constant; a: amplitude of the cupula; ν: is the frequency of the cupula; t: time from the moment the cupula has passed the equilibrium position. The expression between brackets is the change in length of the hair ($\varkappa$), calculated according to Pythagoras. The original length of the hair is supposed to be *one* here and in the calculations below.

Since we are especially interested in the amplitude of the voltage, it is sufficient to calculate x for the maximum deviation of the cupula.

From fig. 30-1 we find for the increase of the length of the hair

$$x = \frac{1 - \cos\alpha}{\cos\alpha} \qquad (3)$$

α is determined by $tang\alpha = a$. This is the case when the hair is in the normal position.

In case it is in an inclined position as in fig. 30-2, we calculate

$$x = \frac{\cos\beta - \cos(\alpha+\beta)}{\cos(\alpha+\beta)} \qquad (4)$$

β is the angle the hair makes with the perpendicular position.

We were interested in the relation between x and a, therefore this relation was calculated. It is given in fig. 31, in which both x and a are expressed in percentages of the original length of the hair.

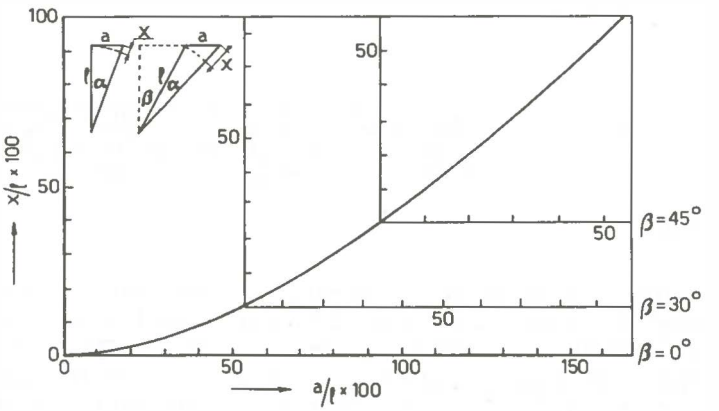


Figure 31. Relation between the amplitude of the cupula and the extent to which the sensory hair is stretched; both a and x are expressed in percentages of the original length l of the hair.
Near the coordinates the value of β is indicated.

For the superimposing experiments I calculated the amplification (e) both for different amplitudes of the higher and of the lower frequency. It was done for the perpendicular and the inclined position of the hair.

In case the hair is in the normal position

$$e = \frac{u}{x_s} = \frac{\cos\delta \cos(\alpha-\varepsilon) - \cos(\alpha+\gamma)}{\cos(\alpha+\gamma)\cos(\alpha-\varepsilon)(1-\cos\delta)} \qquad (5)$$

Here the angles are determined by a_B and a_S.

If the hair is in an inclined position and $\frac{1}{\cos\beta}$ is used for the original length of the hair, we calculate

$$e = \frac{u}{x_s} = \frac{\sin\{\beta+\alpha-\frac{1}{2}(\varepsilon-\gamma)\}\sin\frac{1}{2}(\varepsilon+\gamma)\cos\beta\cos(\beta+\delta)}{\sin(\beta+\frac{1}{2}\delta)\sin\frac{1}{2}\delta\cos(\beta+\alpha+\gamma)\cos(\beta+\alpha-\varepsilon)} \qquad (6)$$

The relation between x and e is given in fig. 32. The values near the curve indicate $a_s/l.100$. The dotted line is the relation we get when the hair is inclined, $\beta = 30^0$ and $a_s/l.100=10$. In that case e will not be more than two.

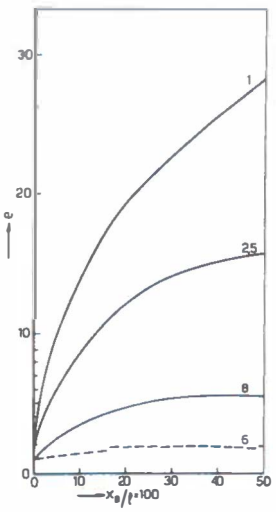


Figure 32. Relation between the stretching effects of the basic vibration and the amplification factor calculated e. The values for $x_S/1.100$ are attached to the curves; the dotted line is obtained for $\beta = 30^o$.

If the hair is already stretched by the B-vibration, the stretching (p) caused by a small displacement in the direction of the equilibrium position of the cupula, differs from the stretching effect (q) caused by a movement in the other direction (see fig. 30-3). This difference depends on the displacement from the equilibrium position. For the quotient p/q we calculate

$$\frac{p}{q} = \frac{\cos(\alpha+\gamma)\{\cos(\alpha-\varepsilon) - \cos\alpha\}}{\cos(\alpha-\varepsilon)\{\cos\alpha - \cos(\alpha+\gamma)\}} \qquad (7)$$

Of importance is the relation between p/q and a_B. In fig. 33 this relation is given for $X_S/l.100 = 1$.

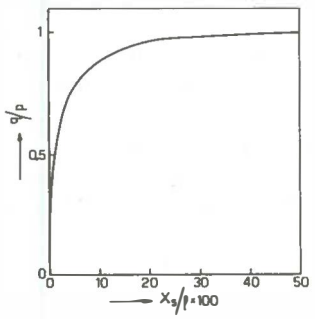


Figure 33. Relation between p/q and x_B in the case $x_S/l.\ 100 = 1$.

The importance of the fig. 31, 32 and 33 is that if the hypothesis $V = k.x$ is correctwe must get similar types of curves for the corresponding voltages of the microphonic effect. So we arrive at the following experimental checks of the hypothesis.

1. The relation between the mechanical and the electrical amplitude must be of a type similar to that given in fig. 31. This relation should be independent of the frequency.
2. According to the hair hypothesis we expect for the phase relation between the driving force and the microphonic effect a curve similar to the one given in fig. 23 and 24 for the displacement.
3. The amplification, A, of the effect of a higher frequency superimposed on a lower one should be studied in detail to check the theoretical consequences in this respect. The influence and the frequency of both the B- and the S-vibration are of special interest. There should be no influence of the frequencies. The quotient I/D has to be investigated in relation to ν_B. We expect a similar relation as found between p/q and ν_B, see fig. 33. According to the theory the "zero-points" (the places on the output curve where the high frequency is not amplified) lay precisely on the positive tops.
4. The electrical behaviour of the cupula during constant displacements will give us an idea to what extent the lateral line organ agrees with the cochlea. The degree in which the cupula must be displaced in order to change the double frequency into the single one, is of special importance to the hair theory.
5. The superposition effect must be studied in the labyrinth, in order to see that the effect is not specific for the lateral line organ.

The experiments described in the next chapter are done with the primary aim to verify the hypothesis $V = k.x$. The

additional assumptions such as "slip" will be discussed in a following chapter.

Some of the points mentioned above have already been studied by de Vries, Jielof and Spoor (1952). To drive the cupula they used alternating watercurrents and in this way they could reach approximately 50 cys/sec. With the magnetic driving method I was able to study the entire frequency characteristic. Moreover, a direct current oscilloscope could be used during my experiments. With this apparatus the microphonic effect of the cupula produced during a constant displacement could be studied.

Chapter VII

QUANTITATIVE RELATION BETWEEN THE MICROPHONIC EFFECT AND THE DISPLACEMENT OF THE CUPULA

In this chapter experiments are described which were made to check the hypothesis $V = k.x$ (see chapter VI). So we assume the voltage to be directly proportional to the extent to which the hairs on the sense cells are stretched by the displacement of the cupula. When this is indeed the case we expect experimental results as predicted in the preceding chapter.

To simplify the discussion the hairs are here assumed to be in the perpendicular position.

The additional assumptions e.g. "slip of the hair", the hair in an inclined position, etc., are discussed in the next chapter.

1. Introduction

In order to investigate the microphonic effect some operations were performed and we want to know whether they influenced the effect in any way.
This question was investigated in the following manner.

On the skin at the outside of the canal, no electric effect could be measured. As soon as a small tip of the pick-up electrode was brought into the canal through a small natural opening in the skin, a microphonic effect could be recorded when the organ was stimulated e.g. by tapping on the table. As de Vries c.s. (1952) remarked, the skin has shielding properties.

Although it is possible to do some experiments without removing any part of the skin, we usually prefer to remove at least the skin caudal from the bone bridge. On doing so we often observed a decrease of the voltage of the microphonic effect. This may be due to: better grounding, less favourable driving situation or damage of the organ.

In order to get some idea which of these possibilities is the most important, the following experiments were done.

The inside of the closed canal was better grounded by putting a short copper wire through the natural hole in the skin. This did not affect the microphonic effect, which was picked up through the opening at the other side of the bone bridge.

Direct grounding by putting a wire-put-on-earth into the canal decreased the microphonic effect by maximal 25%.

When the skin caudal from the first bone bridge was removed very carefully the voltage of the microphonic effect did not decrease. Entire removal of the skin and the bone bridge was always accompanied by a decrease of at least 50%. This operation inevitably implies pulling of the cupula. When some days after the removal of the bridge the cupula was studied again with the magnetic driving method, I could sometimes record the same maximum voltage (saturation value, see below) as observed before the operation.

Consequently the mechanical impairment is more important than the grounding effect. Moreover the damage brought about by the operation will heal. Removal of the skin between the first and second bone bridge offers the best situation for the study of the lateral line organ, because in this case the operation can be carried out without impairing the organ. This situation, whenever possible, was used in the quantitative studies; it will be called "*the nearly natural situation of the lateral line organ*". In some experiments the microphonic effect of the neighbouring cupulae interfered with the effect of the cupula under study. These were then destroyed. When a free organ had a microphonic effect of e. g. 300 μV, at a certain stimulus, a similar value was found four months afterwards. So the unnatural situation of the organ does not seem to be harmful to the source of the microphonic effect.

In aid of the magnetic driving method a small magnet was pinched on the cupula. The question was whether this influenced the microphonic effect. In some cases I compared the voltage curve of the microphonic effect of the cupula driven by alternating water-currents before, during, and after mounting of the magnet on the organ. No difference in the voltage curve could be observed with respect to the wave form, the amplitude and the phase.

A difference in amplitude of the cupula is the most important difference between the organ under the bone bridge and the free organ. To obtain a similar voltage the displacements in the nearly natural situation were usually smaller than one micron whereas with the free cupulae the amplitudes of the organs were several times larger for a similar voltage. This may be a mechanical consequence of a change in the stiffness of the cupula, but we do not know whether the mechanism producing the microphonic effect is influenced. Care must be taken in interpreting the measurements from free cupulae, however.

2. Electric effects with the cupula in resting condition

As stated in the preceding chapter the source of the microphonic effect must be sought in the sense cells. De Vries c.s. found that removal of 2/3 of the cupula need not influence the microphonic effect. They also observed that the sign of the potential is reversed when the electrode is displaced from above to below the organ. I have tried to localise the source of the battery in an other way. A small micro-electrode was thrust down the cupula in the direction of the sense epithelium and the change of the d. c. -potential measured. To reduce the mechanical injury, capillaries with a tip diameter smaller than one micron were used. The observed change of the d. c. -potential is given in fig. 34.

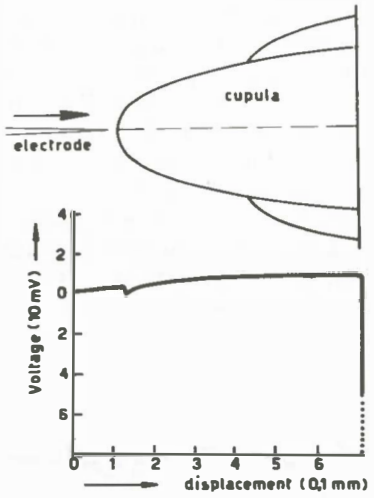


Figure 34. Change of the d.c-potential when a micro-electrode is moved through the cupula as indicated in the upper figure. The small deviation of the voltage curve occurs when the electrode penetrates the cupula and the large jump is observed at the height of the sense epithelium.

As long as the electrode moves in the cupula a small change of the potential is observed. As soon as we are in the vicinity of the sense cells or perhaps within these, we see a considerable change of the potential. The exact value could not be measured, but it is of the order of 100 mV. Von Békésy (1952) obtained similar results in the cochlea of the guinea pig.

When the cupula is not vibrating, there will be no current if the resistance R_1 in figure 28 is infinite. When in this case the electrode is moved up and down close to the cupula (in fig. 29 E moves along EF) no change of the potential difference between the pick-up electrode and the earthed electrode will be observed.

When this experiment is made we observe a change of the d. c. -potential if the electrode is moved close to the cupula. Consequently the resistance R1 in the resting cupula is not infinite. The direction of the current corresponds with what we expected on the ground of the model of fig. 29. No shift is seen when the same experiment is carried out at a distance of 1,5 mm from the organ but still in the canal.

3. Microphonic effect during constant displacements of the cupula

We have assumed V = k. x and in this chapter we suppose in principle that the hair is rigidly connected with the cupula. If these assumptions are correct we may expect a voltage curve corresponding with the constant displacement of the cupula. With the magnetic driving method it is easy to keep the cupula out of this equilibrium position. In this case the current through the coil corresponds with the displacement of the cupula, assuming that it does not creep (see chapter V).

In chapter V we have seen that when an oscillator is suddenly pulled out of its equilibrium position by a constant force, it will make some oscillations round its final position.

The same phenomenon is observed when the oscillator is released from the inclined position. The number of the oscillations depends on the damping. When the cupula oscillates this will be seen in the electric effect of the organ.

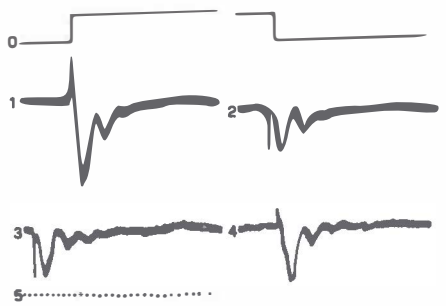


Figure 35. Change of potential during constant displacements of the cupula. Curve 0 indicates the current through the coil. Curves 1 and 3 the course of the potential during displacement from the equilibrium position; 2 and 4 after the release from the inclined position. In experiment 3 reversal of the current. The short peak at the beginning of the deviation is not a microphonic effect but a transient effect of the coil. The course of the curves is the same; the difference in amplitude between 1 and the other curves will be due to a change in the driving situation. Curve 5 timemarks at an interval of 1/15 sec.

Fig. 35 gives some oscillograms of the microphonic effect during which the cupula was pulled out of its equilibrium condition and released after some time.

In these figures we see that the course of the electric effect of the organ does not correspond with the current through

the driving coil. When the cupula is still inclined there is, contrary to our expectations, no measurable microphonic effect.

In both cases, pulled out and released, the oscillations are damped out very soon and the damping, though not critical, is nearly so.

It is remarkable that in both cases the frequency of the oscillations is the same, about 100. This is near the resonance frequency calculated by de Vries (1952). However, in case the cupula is released from an inclined position it vibrates round its equilibrium position and consequently the hair is stretched twice each vibration period. When the organ is pulled out of the equilibrium position it is evident that the hair is stretched once each vibration period (see fig. 27).

Consequently both the course of the d. c. -potential and the equality of the frequencies are not in agreement with the assumptions we have made. They can be explained by the slip effect, as will be discussed in the next chapter.

4. The microphonic effect of the vibrating cupula

4.1. *The influence of the frequency*

When the amplitude of the force is kept constant the voltage of the microphonic effect of the lateral line organ depends on the frequency applied. The resonance frequency of the cupula is about 100 cps (see chapter V). In case the microphonic effect only depends on the amplitude of the cupula ($V = k.x$) we expect to find a frequency characteristic of the same form as the mechanical response curve of the cupula (figs. 23 and 24).

The frequency characteristic of the microphonic effect has been determined in two ways. First by measuring the amplitude of the microphonic effect when the amplitude of the driving force was kept constant (by applying the same current through the driving coil) whereas the frequency was varied. In this way the equal force curves of fig. 36 were obtained.

The variations in the current through the coil can be measured more accurately than those of the voltage of the microphonic effect, because at small amplitudes the voltage is of the same order as the noise. In the other method the amplitude of the voltage of the microphonic effect is kept constant and the frequency and the amplitude of current through the coil regulated; the equal response curves of fig. 37 are thus obtained.

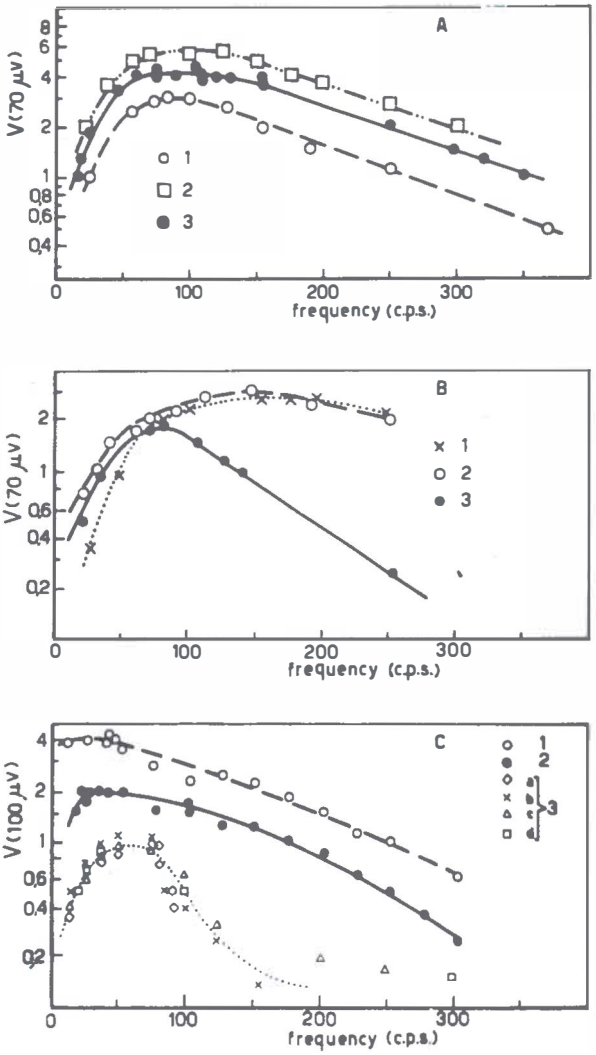


Figure 36. Relation between frequency and voltage of the microphonic effect at constant current through the coil (equal force curves).

A. Curve 1: fish 67, free cupula two days after removal of the bridge. Curve 2: as 1, but 6 days after operation. Curve 3: fish 87, magnet on skin.

B. Curve 1: fish 72, Curve 2: fish 73, Curve 3: fish 86. In these fish magnet on the skin.

C. Curves 1 and 2, magnet on the skin, fish 127. The input in 1 is six times that used in 2. Curve 3: fish 99, a and b cupula above, and c and d below the eye.

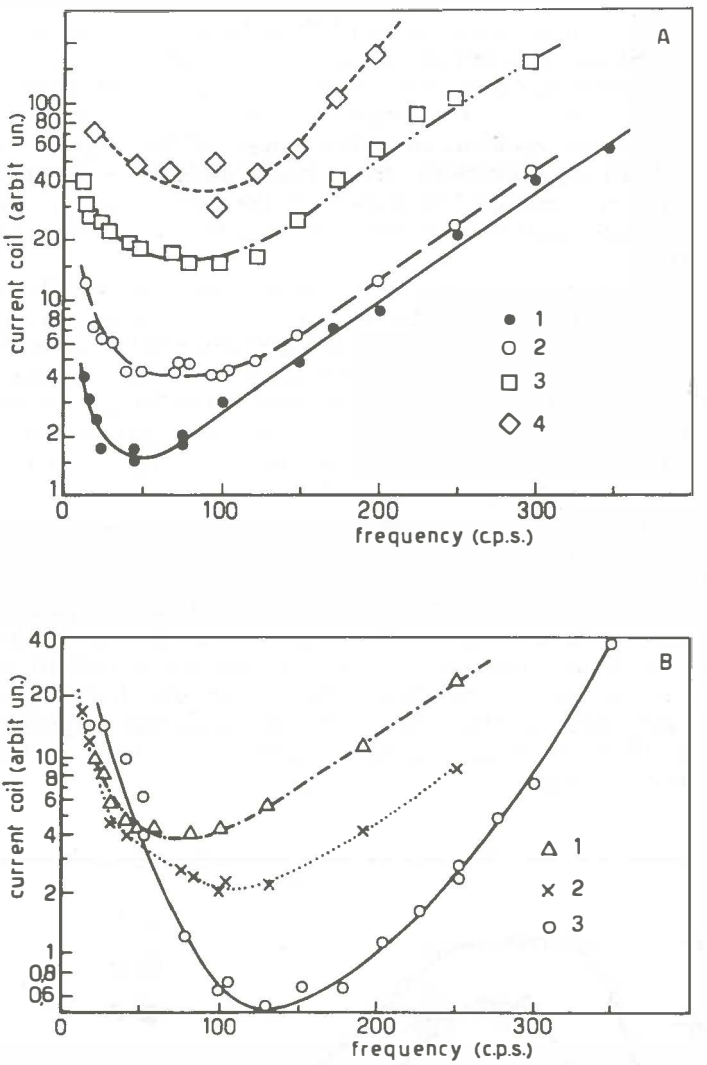


Figure 37. Relation between the frequency and the current through the driving coil necessary to obtain a constant voltage of the microphonic effect (equal response curves). A. Curve 1, fish 112, for V = 75 μV. Curves 2, 3 and 4 for fish 119 at respectively 25, 75 and 150 μV and the cupula in the nearly natural situation.

In fig. 37 curve 1 and 2 is the equal response curve of an organ on which a small magnet was mounted, but which was further left entirely intact. Through a natural hole in the skin the pick-up electrode is brought near the cupula. We see in this figure that the broad top of the curve lies at about 50-150 cps; this is in the region of the resonance frequency of the cupula. It should be kept in mind, however that here we are

not dealing with a free cupula but with a more complex system, consisting of a cupula in a canal, covered by the skin. Now the elasticity arises from the skin plus the cupula. The moving-mass is then the mass of the fluid in the canal (at both sides of the cupula) plus the mass of the cupula. So it is more or less by accident that the resonance frequency is equal to the resonance frequency of the free cupula. We have no direct observations of the resonance frequency of the intact system.

At a low voltage of the microphonic effect, 25 μV, the top of the curves extends between 50 and 200 cps. At greater voltages the top of the frequency characteristic is more pronounced. The removal of the skin and the bone bridge has no consequences for the frequency characteristic, as is shown in fig. 38 curves 1 and 2. We see that the curve determined some days after the removal of the bone bridge resembles the curve of the organ when not prepared free.

In fig. 39 the frequency characteristics of four cupulae, all in the natural situation, are compared. This figure is normalised by adjusting the vertical scale in such a way that the amplitude of V at the frequency at which the organ is most sensitive is taken to be one. There is a spread of the top of the curves. I am not quite sure of the explanation of this. Animals in a poor condition often have the top in the lower region, and this is then the case with all the cupulae on the head (fig. 36-3 curve 1). It may be due to a change in the stiffness of the cupulae.

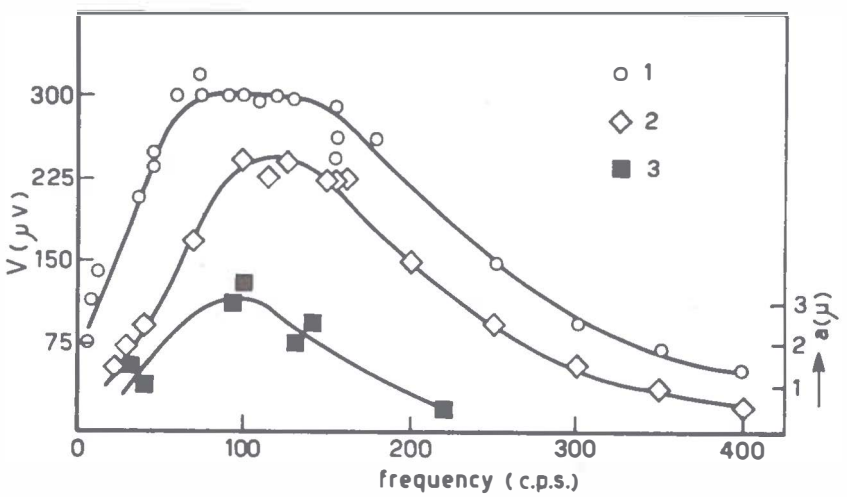


Figure 38. Mechanical and electrical frequency characteristics (equal force curves) of fish 81; 1. magnet on the skin; 2. bone bridge removed and magnet on the cupula; and 3. amplitude of the cupula under identificial conditions as 2.

In nearly all the curves we see an exponential decrease towards the higher frequencies (see fig. 36 and 37).

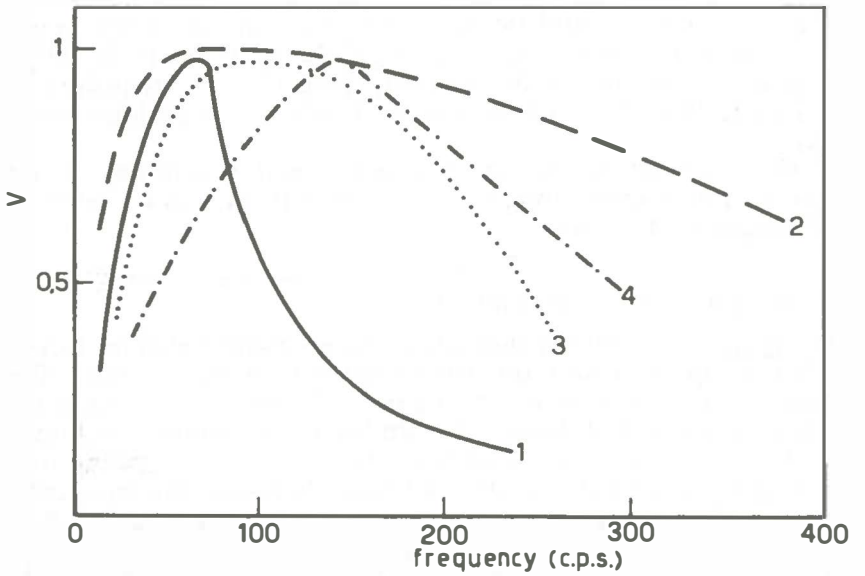


Figure 39. Normalised equal force curves of 4 cupulae in the natural situation.

From the measurements concerning the mechanics of the organ we have learnt that in the nearly natural situation the cupula is about critically damped. This means (see figure 22 curve 6) that on changing from $1/2\ \nu_o$ to $2\ \nu_o$ the amplitude decreases with a factor 4. Anticipating the discussion and the results of the next section (4.2) this would imply that the voltage of the microphonic effect would also decrease a factor 4. Actually the output at these frequencies is nearly the same. This discrepancy demonstrated itself already qualitatively by the consideration that in the case of critical damping there should be no maximum in the frequency characteristic, whereas a broad maximum is very evident.

When the system is supposed to be less than critically damped the decrease of the amplitude above the resonance frequency is greater and the difficulties in explaining the course of the experimental curves, are increased.

In the case of fig. 38 there is a fairly close resemblance between the mechanical and the electrical response curve. However, the maximum of the mechanical curve is below the maximum of the electrical. Here the cupula was free and the voltage was in the order of the maximum value we could obtain from this organ, which means that in this case cellular properties became important. I think that this figure is less typical than figs. 36 and 37.

About 1000 cps was the highest frequency at which we obtained a microphonic effect. In some organs 500 cps was the maximum. Sometimes at the higher frequencies, the voltage curve had the same frequency as the vibration applied.

The conclusion must be that when the cupula is not prepared free, the course of the frequency characteristic is difficult to explain by means of the assumptions $V = k.x$ together with the notion that the hair is perpendicular to the sense epithelium.

If the hair is in an inclined position x increases proportional to a and then some of the difficulties can be overcome (see chapter VIII section 3).

4.2. *Relation between a and V*

In fig. 26 it is shown that there is a linear relation between the force applied and the displacement of the cupula. These measurements are done at greater displacements than those we are dealing with here, but we have no reasons to suppose that the same will not hold good for smaller displacements. Consequently as far as the relation between the mechanical and electrical amplitude is concerned at a given frequency it suffices to give the relation between the amplitude of the force and the voltage of the microphonic effect. A great number of such input-output curves have been made and some characteristics are given in figs. 40, 47 and 51.

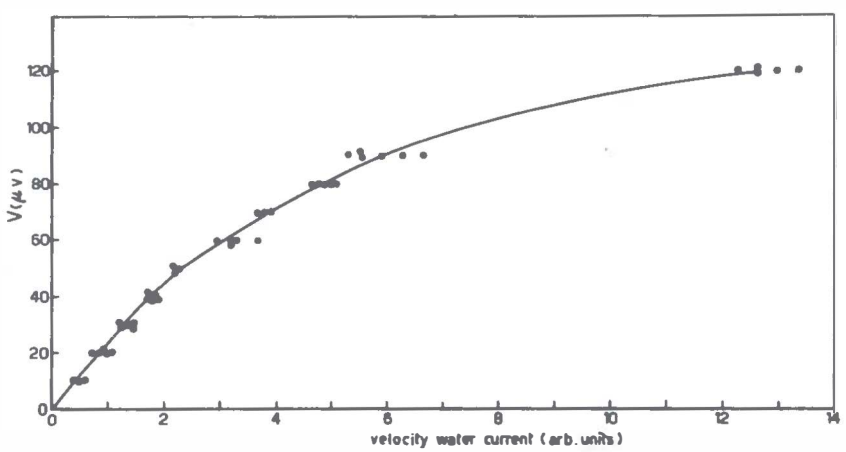


Figure 40. Input-output of a free cupula at 80 cps.

These are of the same type as already described by de Vries, Jielof and Spoor (1952). The first part of the curve shows a linear reaction between the force and V. At higher values of the amplitudes of the force V hardly increases; this saturation value varies with the frequency.

At least in the experiments with the cupula in the nearly natural situation, the amplitude of the cupula is very small and much less than 1 μ; this has already been described in chapter V. We are interested in the increase in the length of the hair, x, and calculate $x = 1 + a^2 - 1$ (assuming the original length of the hair to be one).

$$1 + x = 1 + \tfrac{1}{2}a^2 + \tfrac{1}{4}a^4 + 1/8a^8 \ldots\ldots$$

Because a < 1/10 we neglect the third and following terms. So

$$x = \tfrac{1}{2}a^2,$$

the change of the length of the hair is proportional to the square of the amplitude of the cupula. This means that on the assumption $V = k.x$ we predicted a concave curve for the relation between the mechanical and electrical response in case the hair is in the perpendicular position. When we assume the hair to be inclined 30° or more with the normal on the direction of movement of the cupula, a linear relation is possible.

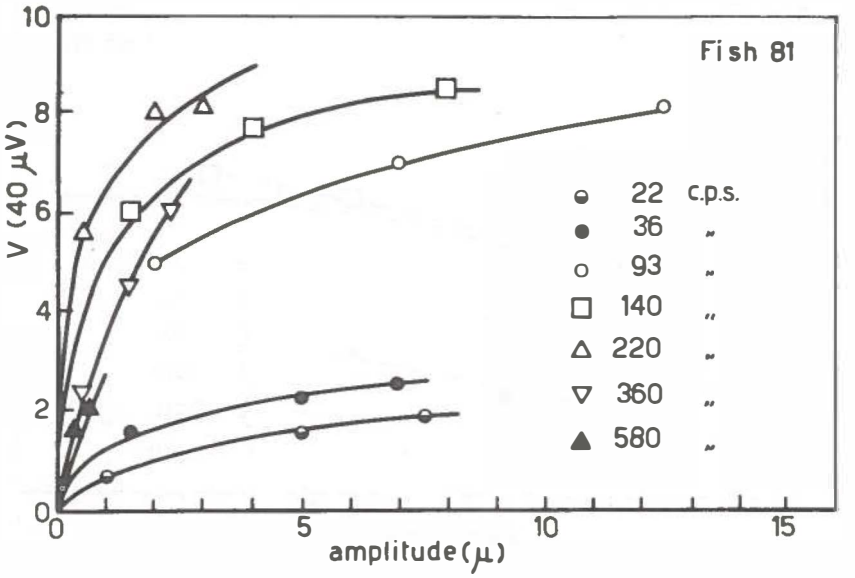


Figure 41. Relation between the amplitude of a free cupula and its microphonic effect at given frequencies. The curves are drawn on the basis of the input-output course of the relation between the current coil and the voltage of the microphonic effect.

The direct relation between a and V at different frequencies can only be studied by measuring a. This relation was determined in the following manner (see also chapter II). The amplitude and the phase of the microphonic effect were measured

with respect to the force. Then the same was done for the displacement of the cupula. To make sure that the situation was not changed in the end, I repeated the electrical measurements.

The relation between the input and the mechanical amplitude is linear (see chapter V). The straight line drawn through the points was used to convert the input-output curve (like figure 40) into a "amplitude-output" curve. Two of these are given in figs. 41 and 42. According to the notion $V = k.x$ the same displacement should give the same voltage independent of the frequency, or, in other words, all the points in these figures ought to lie on the same curve. Again the experimental results conflict with the assumption $V = k.x$. De Vries c.s. (1952) did not observe an influence of the frequency. However, as stated before they could only study this matter in a narrow frequency range, viz. between 10 and 50 cps. In this range our measurements likewise do not show a marked influence of the frequency. For an extensive discussion see chapter VIII section 5.2.

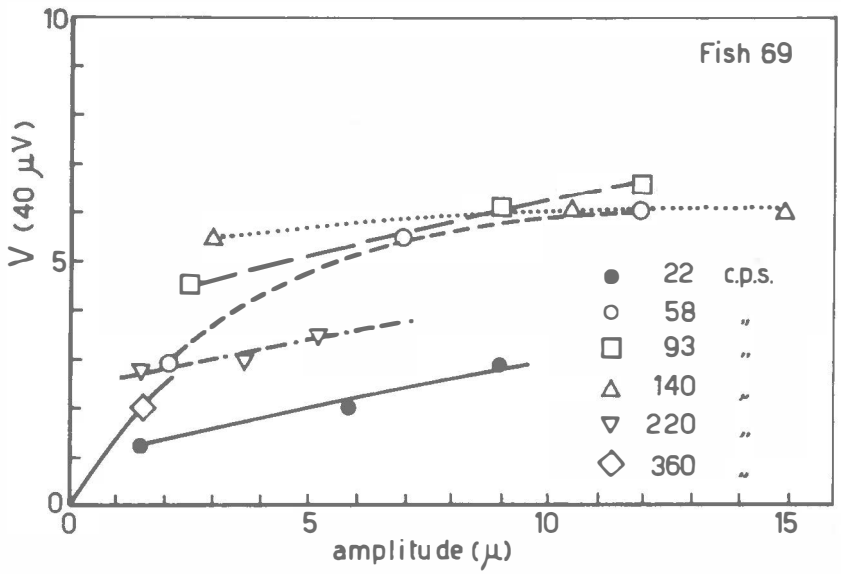


Figure 42. Similar relation as in fig. 41.

4.3. *The difference in phase between the motion of the cupula and its electric effect*

If the voltage of the microphonic effect is related to the extent to which the hair is stretched, so $V = k.x$, this has

consequences for the phase of voltage with respect to the movement of the cupula.

a. We expect the maximum voltage at the moment of maximum displacement. This can be checked by measuring the difference in phase of both the mechanical and electrical response with respect to the force and calculate from these measurements the phase difference between the mechanical and electrical response; this difference is called φ.

b. In chapter V we saw that the phase difference between the force and the displacement of the cupula changed 180^{o} at most, when the frequency varied from 10 to 300 cps. Therefore, according to $V = k.x$, the phase of the microphonic effect may not change more than 180^{o}.

The second type of experiment is much simpler than the measurements of the actual difference in phase φ.

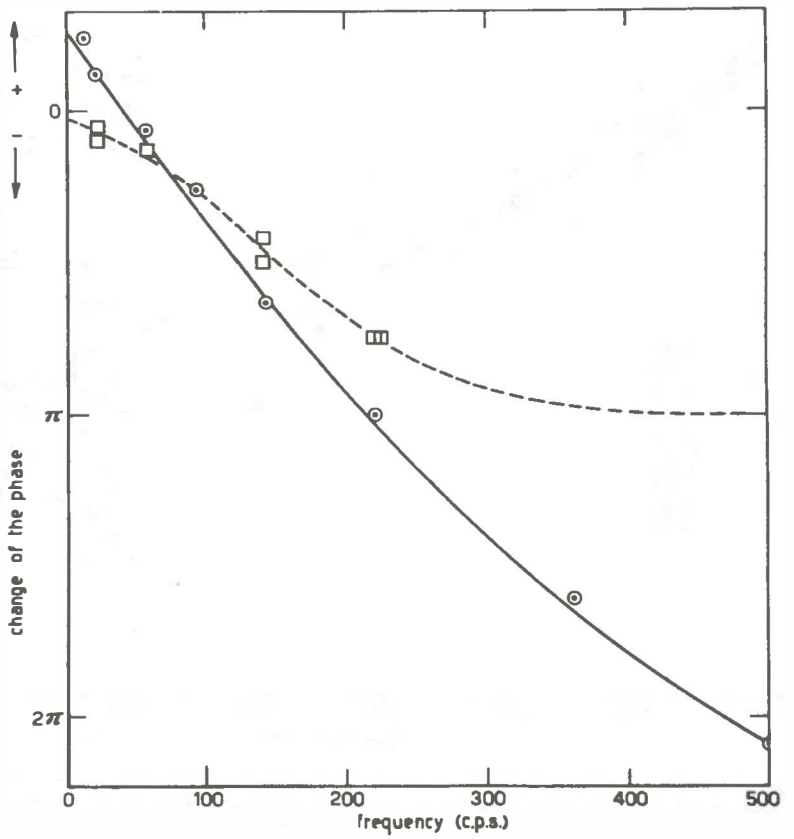


Figure 43. Relation between the frequency applied and difference in phase between the maximum of the force and the maximum displacement (dotted line); this curve is the one we expected for a harmonic oscillator (see chapter V). The full line gives the same relation for the maximum value of the voltage of the microphonic effect.

In fig. 43 the phase difference of both the mechanical and electrical response with respect to the force is plotted against the frequency. We see that at low frequencies the electrical effect is in phase ahead of the mechanical response, whereas at high frequencies there is a lag in phase.

From this kind of diagrams fig. 44 was prepared. For nine animals this figure gives the relation between the frequency and φ. Though the spread of the points is considerable, there is a similar tendency in all fishes, viz. a linear relation between φ and the frequency. At low frequencies the φ is maximal 45^{o} ahead. De Vries c.s. (1952) found a same tendency at the low frequencies as I did, but they observed greater values, about 70^{o}.

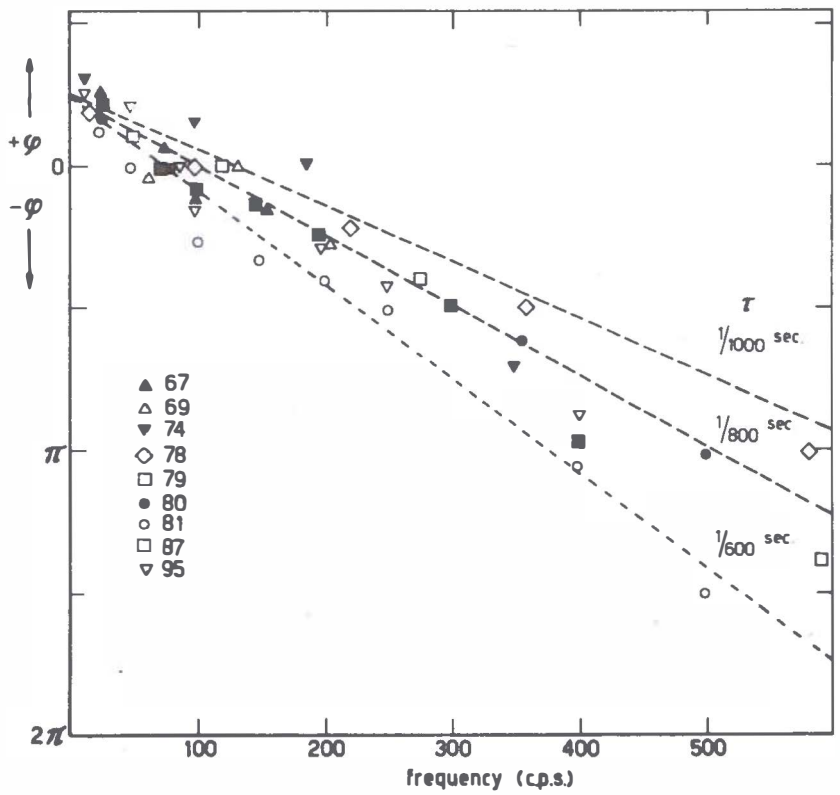


Figure 44. For nine animals (numbers are indicated near the marks) the interrelation of the frequency and the difference in phase between the mechanical and electrical response (φ). The dotted lines indicate the course we calculate in case a latent time τ is introduced (see chapter VIII-2.2).

For a cupula in the natural situation fig. 45 gives the relation between the frequency and the amount to which the phase of the microphonic effect is changed. For all the cupulae thus

investigated a similar type of curve was obtained. In the range in which the phase of the mechanical response changes with π, the electrical changes with 2π.

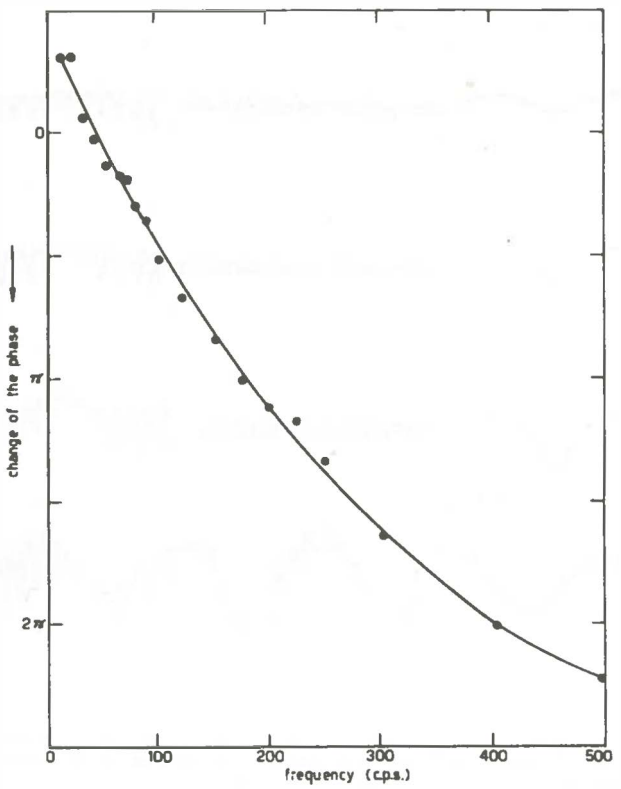


Figure 45. For a cupula in the natural situation, the phase difference between the driving force and the microphonic effect as a function of the frequency.

Once more these experimental results are not in keeping with our assumption that the voltage would be in proportion to the extent to which the hair is stretched.

5. Superposition effect

As already pointed out in chapter V de Vries discovered the superposition effect – the amplification of the output of a high frequent vibration when superimposed on a lower one – as

a consequence of his hair hypothesis. We have seen in this effect a good support to the correctness of the hypothesis. We shall now turn our especial attention to this superposition effect in relation to the assumption $V = k.x$.

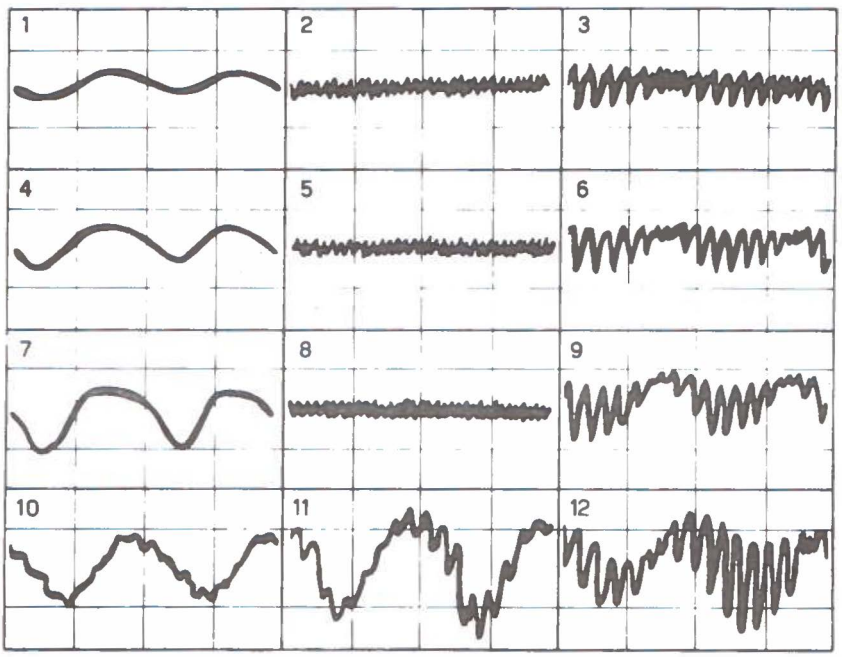


Figure 46. Oscillograms of the superposition effect. In 1, 4 and 7 the effect of different amplitudes of the basic vibration (40 cps). The superimposed vibration (400 cps) is kept constant (2, 5 and 8). The combination of the two vibrations in 3, 6 and 9 (e.g. 3 = 1 + 2). In 10, 11 and 12 the effect of an increase of the amplitude of the superimposed vibration at a constant amplitude of the basic vibration.

Some oscillograms of the effect are reproduced in fig. 46. Note that the single frequency of the S-vibration tends to become double in the positive tops and that especially at relatively great amplitudes of the S-vibration there is a tendency to a frequency doubling in the "amplification phase". The symbols for the different components have already been described in chapter V, fig. 30. The relations between the different components of the microphonic effect during superposition as found in experiments are discussed below separately.

5.1. *The relation between V and A*

For the determination of this relation we kept the amplitude of the S-vibration constant and varied that of the basic

one. From pictures like those in fig. 46 we determined W and plotted A against V ($A = \frac{W}{V_S}$). In fig. 47 we see that this relation has a maximum about the value of V_S, at which the input-output curve (fig. 47b) shows a deviation from the linear relation. Fig. 48 demonstrates a similar relation, found at other fish. In the case of fig. 47 the two negative peaks of the B-vibration had a different amplitude, as indicated in the figure. The origin of this asymmetric wave form was unknown here. But setting aside its cause we see in this figure that the relation between A and V_B is the same for both peaks.

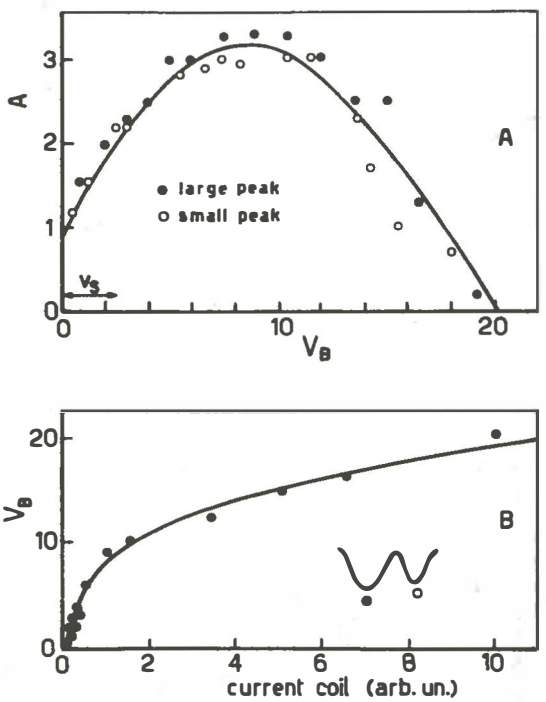


Figure 47. Relation between the voltage of the microphonic effect caused by the basic vibration (44 cps) and the amplification of the superimposed vibration (370 cps). The input-output curve of the basic vibration is indicated in fig.B. The wave form was an asymmetric one as indicated in the figure by different marks.

It is remarkable that this superposition effect appeared in the linear part of the input-output. During the superposition the small amplitude of the higher vibration causes a larger amplitude than in case it is given alone; hence the superposition effect is a non-linear effect. The decrease in amplification might be due to the same effect as the maximum value of V_B at the higher amplitude of the cupula. When we leave

out this decrease of A the course of the curve resembles the theoretical one of fig. 32; the observed amplification is smaller than the calculated one.

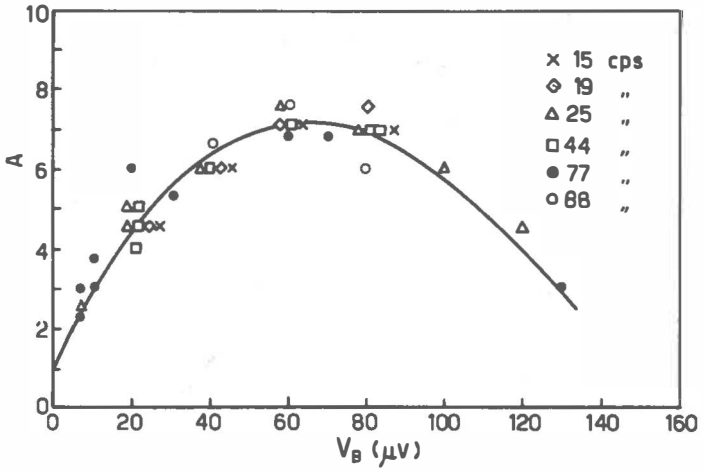


Figure 48. Relation between the amplification of the superimposed vibration (440 cps) and voltage of the basic vibration at different frequencies of this vibration.

5.2. *The relation between V_B and A*

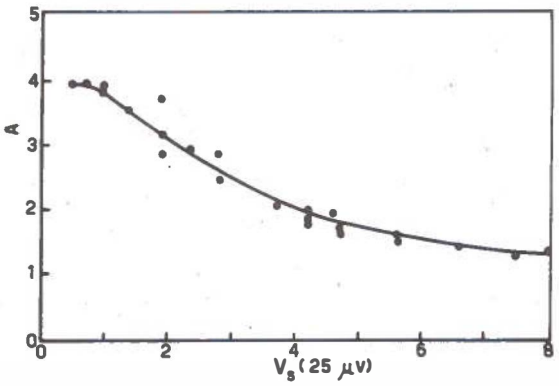


Figure 49. Relation between amplification factor A and the voltage of the superimposed vibration at a fixed value of the basic vibration.

This relation is shown in fig. 49 and fig. 50. We see that on increasing the amplitude of the S-vibration at a fixed V_B, the amplification decreases. At great amplitudes of both vibrations, A is not more than two. According to $V = k.x$ we expected with the increasing of V_S a similar decrease of A.

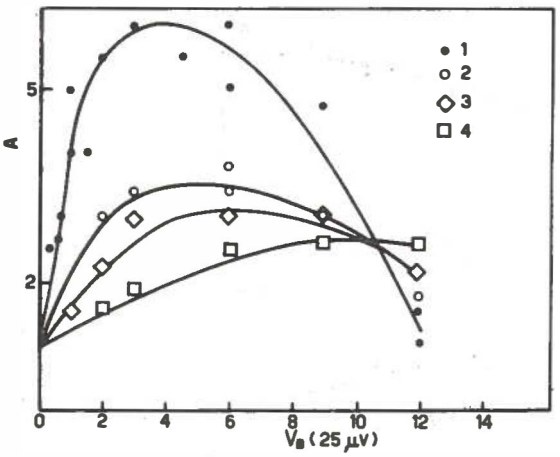


Figure 50. The relation between the voltage of the basic vibration and the amplification of the superimposed vibration at 25 V; 3. nearly 100 V. 4 gives the values obtained at the end of the experiments.

5.3. *The influence of the frequency of the B-vibration on A*

As is shown in fig. 48 a marked influence of the frequency of the B-vibration was not found. In only one case such an influence was observed; at 88 cps. I found for the maximum $A = 5$, whereas at 44 cps it was not more than 4. In the other cases I obtained figures like fig. 48; so no influence of the frequency of the B-vibration. These results support the notion $V = k.x$ according to which there should be no influence of the frequency. It is, however, hardly possible to study the influence of the frequency of the B-vibration above 80 cps because the difference between the two frequencies is then too small to make careful measurement possible.

5.4. *The influence of the frequency of the S-vibration on A*

In order to study this effect the frequency and the amplitude of the B-vibration were kept constant. The amplitude of V_B is indicated by an arrow in fig. 51. Of the S-vibration the V_S was the same but its frequency was varied. There is a pronounced influence of the frequency of the superimposed vibration in fig. 51. The amplification is optimal at about 300-400 cps. This result is not in agreement with our starting-hypothesis $V = k.x$, according to which only the amplitude is essential.

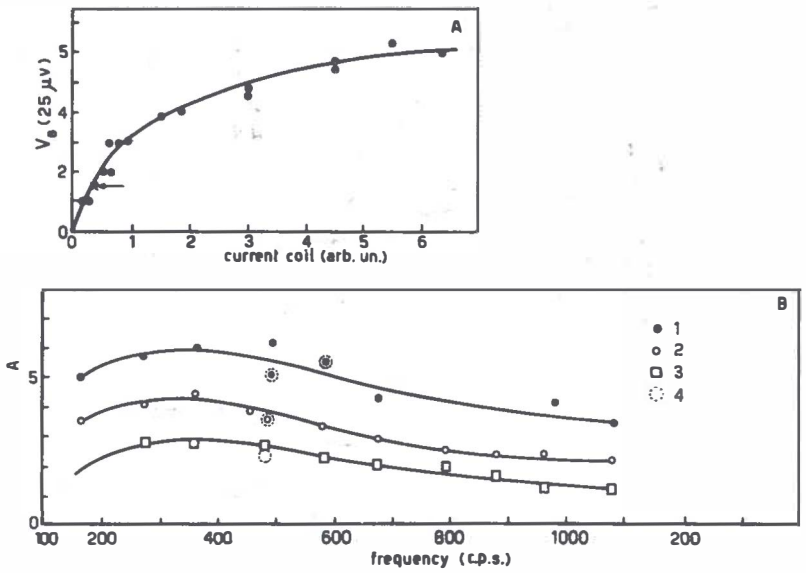


Figure 51. A. Input-output curve at 44 cps. B. Relation between the frequency and the amplification factor A at a fixed value of V_B (indicated by the arrow in fig. A) for three values of V_S; 1. about 20 μV; 2. 50 μV; 3. nearly 100 μV. 4 gives the values obtained at the end of the experiments.

5.5. *The quotient D/I*

In fig. 46 it can be seen that in the negative peaks produced by the B-vibration the voltage of the S-vibration does not move exactly around the voltage curve of the B-vibration. When during the experiments V_S was increased it looked as if the negative peaks of the B-vibration were filled by the S-vibration. So the effect in an upward direction (D) is greater than downward (I). This effect was studied in more detail.

First an oscillogram with synchronised time base was made (see figs. 46 and 52-2). Secondly an oscillogram with a slow time base was recorded of the B-vibration on which for a moment the S-vibration was superimposed (see fig. 52-3).

Because of the slow horizontal rate of the beam the separate oscillations cannot be seen in these recordings but only the enveloping figure. In these recordings the increase of the potential, i.e. I, was measured. As $D + I$ (fig. 52-4) and I was known, D/I could be calculated. The relation between this quotient and V_B is given for different frequencies in fig. 53. We see that with the increase of V_B the value of D/I decreases. In chapter V, fig. 33, we saw that the theory predicted quite the contrary. Here again the experimental results are incompatible with the assumption $V = k.x$.

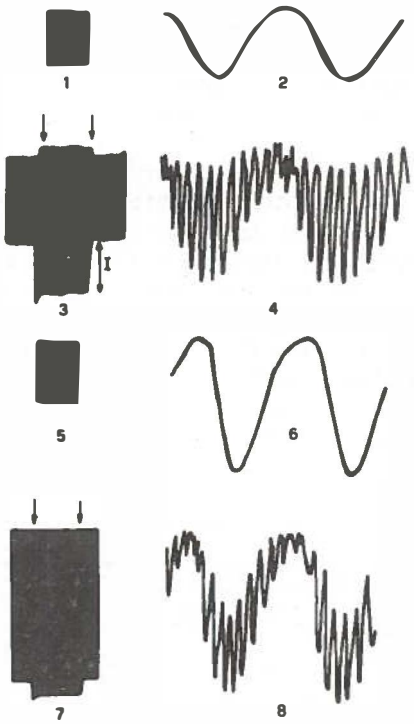


Figure 52. Oscillograms of the superposition effect. Recordings 1-7 were taken with a slow time sweep. 1. voltage of the superimposed vibration (250 cps). 2. synchronised voltage curve of the basic vibration (25 cps). 3. recording of the basic vibration; between the arrows the superimposed vibration is also applied. 4. same as 3 but basic vibration is synchronysed. 5, 6, 7, and 8 are similar recordings at a larger amplitude of the basic vibration.

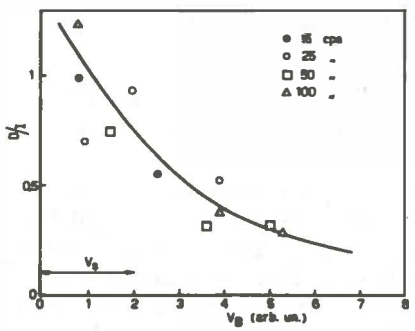


Figure 53. Relation between D/I (see fig. 30) and V_B.

5.6. *Zero-points of the superposition effect*

The moment at which the amplification is smallest (the "zero-point") does not coincide exactly with the positive top (see fig. 46-5). When the amplitude of the superimposed vibration is diminished the zero-pointed becomes a zero line,

which extends more in the direction of the negative than in the direction of the positive top (see fig. 46). The frequency of the S-vibration does not influence the zero-point, that of the ground vibration sometimes does. For the experimental results see table 2.

The zero-point was nearly always 15-30° ahead of the positive top. Just after the operation it sometimes lags in phase behind the positive top, but some days afterwards it was ahead. So normally the zero-point is ahead of the positive top; when this is not the case it may result from some impairment of the cupula or of the sense cells.

According to the hypothesis $V = k.x$ and the assumption the hair in perpendicular position the zero-point must be on the positive top; at this moment the hair is in perpendicular position for the B-vibration and only the stretching effect of the S-vibration occurs.

Table 2

fish	date	45 cps		35 cps		15 cps		difference in phase between zero-point and positive top
		ahead	lag	ahead	lag	ahead	lag	
31	12/8	15-30	-	15-30	-	30	-	free cupula, operation 28/7
31	19/8	0-5	-	0-5	-	0-10	-	V_B decreased with about 50% compared with 12/8
36	5/7	0	0	-	15-30	-	30	free cupula
36	6/7	0	0	-	-	-	0-15	free cupula
37	10/7	10-15	-	0-15	-	0-7	-	cupula still under bone bridge
38	10/7	0-15	-	-	-	0	0	id.
38	11/7	30	-	20	-	15	-	id.
38	14/7	15	-	-	15	-	-	id.
39	24/7	30	-	-	-	-	-	id.
39	25/7	15	-	15	-	15	-	id.
39	28/7	7-15	-	-	-	-	-	skin removed

6. The electric effects when the cupula does not vibrate round its equilibrium position

As stated in 1952, as a consequence of the hair theory the double frequency of the electric effect of the cupula changes to the single frequency when the cupula does not vibrate round its equilibrium position. In this case the hairs are stretched only once each period. At the time we saw that, in order to obtain the single frequency, the cupula must be displaced considerably further than its vibration amplitude. This was even more easily demonstrated by means of the magnetic driving method (see fig. 54). It appeared that at a displacement of 5-7 times the amplitude, the frequency was single. At a displacement of 2-3 times the vibration amplitude there was, apart from a change in phase, no influence on the voltage curve.

In fig. 55 some recordings with a slow time scale are re-

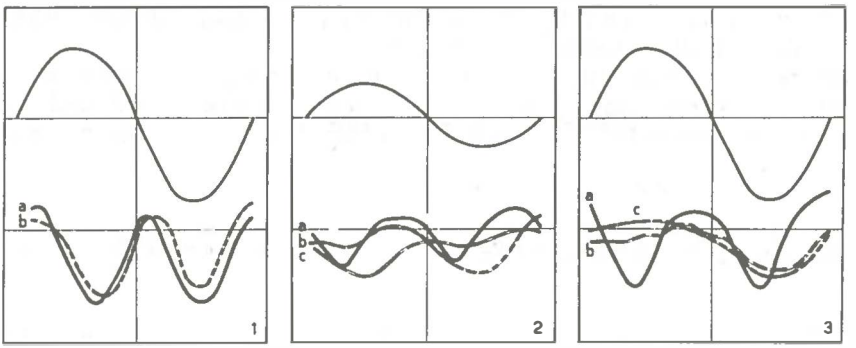


Figure 54. Course of the potential during one vibration period when the cupula is displaced out of its equilibrium position. The upper curve indicates the voltage on the driving coil. Curve a gives the normal situation.
Fig. 1: b Frequency 20 cps. Amplitude of the cupula 2 μ. Constant displacement 4 μ.
Fig. 2: b Frequency 80 cps. Amplitude about 1 μ. Constant displacement 6–7 μ in the direction of the coil. in 1 c the same constant displacement away from the coil.
Fig. 3: Frequency 80 cps. Amplitude of the cupula 4 μ. In b constant displacement 20 μ and in c about 25 μ.

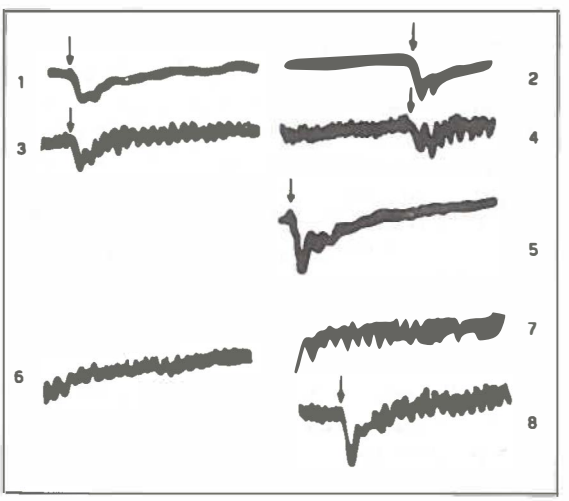


Figure 55. The course of the microphonic effect when a free cupula is displaced out of the equilibrium position (1) and when it is released from the inclined position (2); the arrows mark the moment at which the force is applied. In 3 and 4 similar recordings but with vibration of the cupula (frequency of the ac-current driving coil 300 cps). Curve 5 conditions as in 2 but at an other cupula; 6 as 3 and 7 and 8 as 4.

produced taken at the moment that the vibrating cupula is displaced by a constant force. Just after the moment the cupula is displaced we see that the frequency of the microphonic effect is the single frequency.

After some time, in some experiments a few, and in others about 10 seconds, the frequency is doubled again.

Moreover we learn from these recordings that it is immaterial whether the cupula is displaced from the equilibrium

position or released from the inclined position; in both cases the same phenomenon is observed.

These experimental results are not in agreement with the simple assumption $V = k.x.$, but they can be explained with the aid of slip effect, as will be pointed out in the next chapter.

7. Superposition effect in the labyrinth of the pigeon

After the findings described above it became evident that the superposition effect does not provide good support for the hair hypothesis as we had thought at the beginning. It is probable that the sense cells themselves are responsible for this effect and not the "mechanical stimulating arrangement" of these cells. Therefore it was of importance to investigate whether the effect also occurs in the labyrinth organs, so as to make sure that the effect is not specific for the lateral line organ of the fish. We, de Vries, Kuiper and Ubbens (1955), succeeded in demonstrating the effect in the labyrinth of the pigeon in the very first experiment performed for this purpose. In the semicircular canals the amplification was of the same order as in the lateral line organ. In the cochlea there was no or hardly any amplification of the voltage of the S-vibration. However, during part of the period of the B-vibration there was no effect of the S-vibration at all, in other words, there was a marked zero-point like we observed in the lateral line organ. Hence the oscillogram of the superposition effect of the semicircular canal and the lateral line organ.

Together with Ubbens a more quantitative analysis of the effect was made. The method was essentially the same as that used for the lateral line organ (see also thesis Ubbens 1955).

7.1. *Semicircular canals*

Fig. 56 gives some oscillograms of the superposition effect in the pigeon. In figs. 57-1 and 2 the relation between A and $_B$ is demonstrated for some pigeons. Here too, we have an optimum curve. The maximum value for A depends on the animal used; it was usually 3, but higher values were often obtained, up to about 10 (see fig. 57-3). Just after the operation the amplification was higher than when the animal had been studied for about half an hour.

Fig. 57-3 shows the relation between A and V_S. The curve has the same course as found for the similar experiment with

the lateral line organ (see fig. 49). The amplification occurs in the linear part of the input-output curve, like in the lateral line organ.

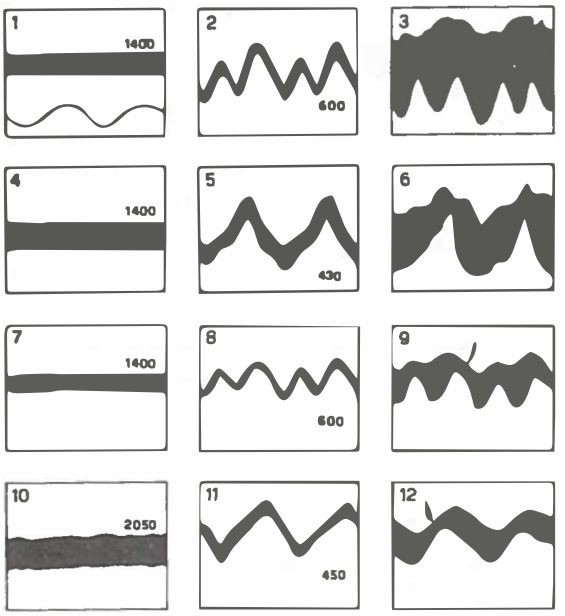


Figure 56. Superposition effect in the microphonic effect of the pigeon. 1-10 of the semicircular canals; 10, 11 and 12 of the cochlea. The frequencies are indicated in the figures. The effect of the combination of the two sounds is given in the figs. 3, 6, 9 and 12 (e.g. 3 = 1 + 2). The time sweep was synchronised for two vibrations of the basic vibration indicated in 1 and the time of exposure in the photographic recording was several seconds.

7.2. *Cochlea*

The superposition effect in the cochlea is demonstrated in the oscillograms of fig. 56-10, 11 and 12. These show a great resemblance to the other figures but the amplification is lacking. The zero-point (marked by an arrow) is mostly in phase ahead of the positive top (about 90°) and at a decrease of the amplitude of the higher vibration, it extends more in the direction of the negative peak than in the opposite direction.

In both, in the cochlea and in the semicircular canals, the frequency of the vibration has no significant influence on the picture of the effect, as long as the frequencies used differ a factor 2 or more. Extirpation of the membrana tympani has no influence on the form of the superposition effect.

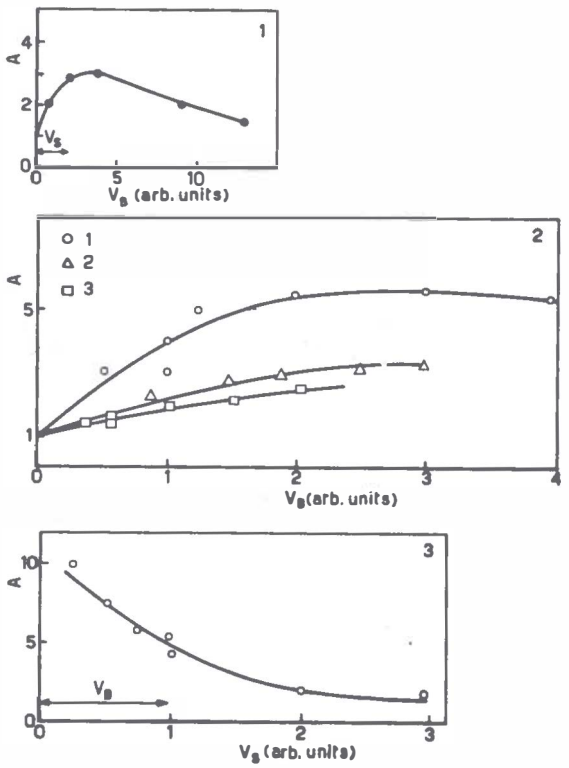


Figure 57. Superposition effect at the labyrinth of the pigeon. The dependence of the amplification factor (A) of the voltage of the basic vibration and of the superimposed vibration. The numbers near the marks indicate V_S in arbitrary units.

8. Conclusion

We started from the notion that the hairs on the sensory cells are in a perpendicular position and that the voltages of the microphonic effect are in proportion with the tension in these hairs ($V = k.x$). We may summarise the experimental results obtained to verify this assumption, by stating that on nearly all the points on which the theory could be checked, the quantitative experimental results are not in agreement with this notion.

1. The most important contradiction is formed by the linear input-output curve and the non linear superposition effect. Without additional assumptions this reaction cannot be explained by one and the same mechanism. In the natural situation when the amplification is very pronounced the amplitude of the cupula is much less than one micron during superposi-

tion. When the electric effect approaches its saturation value we found in the experiments amplitudes of about one micron (see chapter V section 4). This means that in the range in which the amplification occurs, the amplitude of the cupula is certainly less than 10% of the original length of the hair, for which we found about 7 microns. Then the theory gives a quadratic relation between the displacement of the cupula and the stretching effect of the hair. Hence the input-output curve has to be a concave curve as a consequence of the assumption $V = k.x$. A linear input-output curve is obtained when the hairs are inclined, but then no amplification occurs (see fig. 32). So curiously enough our conclusion must be that the superposition effect is not an experimental support for the hypothesis.

The occurrence of the doubling of the frequency is easily explained by the model. It is, however, necessary to assume that the hair can "slip", to give a full explanation of the experimental results we obtained during constant displacements of the cupula.

Stationary displacements of the cupula are not accompanied by a microphonic effect. On this point the organ differs from the organ of Corti (von Békésy, 1951).

2. It appeared that not only the amplitude of the cupula must be brought into account. At different frequencies but the same voltage of the microphonic effect the amplitude of the cupula was not the same. The maximum in the frequency characteristic of the microphonic effect is not in agreement with the critical damping of the lateral line organ.

3. By the hair theory in its original form it is impossible to explain the course of the relation between the frequency and the phase difference between the mechanical and electrical response of the lateral line organ.

It is of importance that in the labyrinth of the pigeon superposition effects occur similar to those found in the lateral line organ of fish.

Chapter VIII

EXTENSIONS OF THE HAIR THEORY

In the foregoing chapter it was proved that the hair theory in its simplest formulation fails to explain several aspects of the experimental results obtained. The two essential assumptions we raised there were:

a. In the resting cupula the hair on the sense cell is in the perpendicular position;
b. the voltage of the microphonic effect is proportional to the extent to which the hair is stretched ($V = k.x$).

This chapter will deal with a few modifications of the assumptions *a* and *b* and with these we will try to overcome some of the discrepancies between theory and experiment.

1. Slip effect

In chapter VI a description was given of our ideas on the connection between the cupula and the hair on the sense cell. It will be clear that this connection should not be seen as rigid coupling but that a slip effect is quite possible.

In the following sections we shall use the terminology described in chapter VII. So we will say that the hair is stretched though actually no stretching of the hair will occur. I wish to emphasize once more that this is merely a matter of terminology. The term "stretching" is only used as an indication of the force applied to the sensory cell. The same is the case with the slip effect we shall discuss in this section. This effect does not necessarily take place at the connection between the hair and the gelatinous substance of the cupula, but is more likely to occur between the various parts of the gelatinous substance. An indication for this is obtained from our study of the mechanics of the cupula (chapter V) where we have seen that the cupula does not behave, as we had expected at first sight, as an elastic body but that it is plastic to a constant force.

When a vibrating cupula was displaced by a constant force the amplitude of the cupula, due to the vibration, was nearly the same as it was before the constant displacement. This holds good as long as the constant displacement is not too large. The cupula seems to be almost ideally elastic to vibration.

Obviously these properties are of biological importance

when the organ is used to detect vibrations. In nature a grain of sand may get into the canal of the lateral line organ and displace the cupula over several microns. But then the organ maintains its sensitivity to vibrations. During the experiments I often observed air bubbles in the closed canals which could be moved through the canal from one side of a bone bridge to the other. This inevitably implies considerable displacements of the cupula but it did not affect the microphonic effect of the cupula below this bridge.

If no slip effect would occur the hairs of the sense cells would be stretched as long as the displacement of the cupula is maintained. This would imply that the cell is continuously stimulated and that at larger displacements the whole system will be destroyed. But at constant displacements of the cupula the sensorium preserves its sensibility to vibrations if the hairs slip back to their initial position.

We will now see if we are able to surmount some of our difficulties in explaining the experimental results by assuming slip. De Vries c.s. (1952) observed that the constant displacement of the cupula necessary to suppress one of the negative peaks induced by a vibration exceeds the amplitude of the organ. They proposed to explain this with a slip effect. The experimental results described in chapter VII support this idea. During constant displacements of the cupula no microphonic effect was obtained (i.e. no constant change of the d.c. potential) and this agrees with the idea that the hair slips back to its initial position.

Moreover it becomes comprehensible that the oscillations in the voltage curve had the same frequency, viz. the proper frequency of the cupula, both when the cupula is displaced from the equilibrium position and when it is released from the inclined position. The hair is stretched at the moment the cupula is displaced. But during this displacement the hair creeps from its inclined position through the gelatine back to its initial position. When the cupula is released the hair is stretched again but now it is inclined in the other direction. Afterwards it creeps back again to the normal position. Consequently when such a slip occurs it is not essential for the cell whether the cupula vibrates around its equilibrium position or away from this. Judging from the decay of the d.c. potential this slip effect is a fairly rapid process; the halftime value of the decrease is of the order of 0,08 sec. in the recordings of fig. 35.

There are some difficulties, however. When we look at the recordings of fig. 55 we see that, when the d.c. component is back at the initial value, the frequency of the microphonic effect by the vibration still has the single frequency. The time it takes to become double again is considerably more than that for the back fall of the d.c. potential.

We have observed a similar behaviour when a cupula is displaced over an amount not large enough to change the double frequency of the microphonic effect into the single. Immediately after the displacement the frequency of the microphonic effect is single, but after a time (varying between one and several seconds) it is double again.

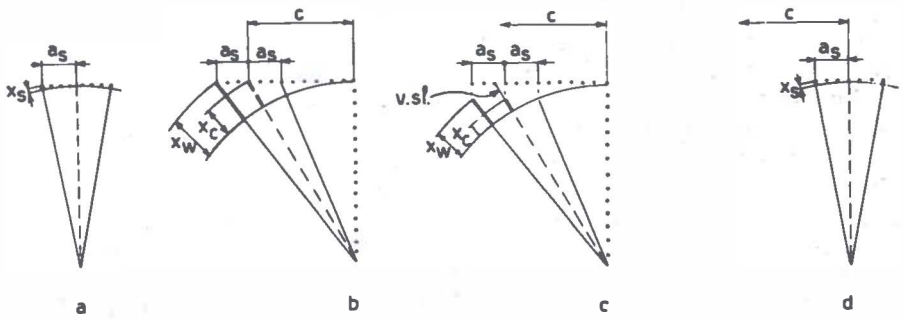


Figure 58. Schematic representation of the vertical and horizontal slip of a hair in the perpendicular position.
a: only a vibration applied with a small amplitude (a_s)
b: cupula displaced an extent c. The hair is now stretched x_w by the vibration and x_c by the constant displacement $x_w > x_s$.
c: vertical slip has occurred and consequently x_c and x_w are decreased.
d: horizontal slip has occurred and hair back in the original position.

To explain those two effects we shall use fig. 58. Immediately after the cupula is displaced the hair is stretched. In consequence of the slip it is back to its initial length after some time. Then the hair is still inclined (fig. 58-c). Moreover we assume that the hair creeps back gradually to its normal position. Hence we discriminate between a *vertical* and a *horizontal slip effect*. A vertical slip effect with a small time constant and a horitontal slip effect with a larger time constant. In the case of vertical slip we suppose that in the gelatinous substance of the cupula, different parts move along each other in a vertical direction. During the horizontal slip a similar process in a horizontal direction occurs.

The vertical slip might be correlated with the rapid back fall of the d.c. potential at constant displacement of the cupula, whereas the horizontal slip is due to the longer time the organ needs to produce, after a constant displacement, a microphonic effect of the double frequency.

No precise measurements are available at the moment but we have gained the impression that the difference between the

velocity of the two forms of slip varies with the animal studied.

According to the idea $V = k.x$ the amplitude of the microphonic effect produced by the vibration must be larger when the hair is in an inclined position than when the hair is in the perpendicular position. In the recordings of fig. 55 this is indeed the case. However, according to the same assumption the difference in amplitude of the microphonic effect before and after the displacement of the cupula ought to depend on the extent to which the cupula is displaced. So far we have not observed such an influence.

The conclusion is that with the aid of the slip effect several difficulties we had in explaining the results obtained at constant displacement can be overcome. On account of general biological considerations such a slip effect seems fairly plausible.

In this chapter we are dealing with mechanical extensions of the hair hypothesis and suppose that the slip occurs at the connection hair-cupula. But it is also possible that the phenomena now explained by slip are partly due to a reaction of the cell itself (see chapter XII).

All the experiments in which the cupula underwent a constant displacement were performed with free cupula. It seems necessary to repeat them with organs in the nearly natural situation.

2. The phase behaviour of the microphonic effect

A striking conflict between the hair hypothesis in its general wording and the experimental results is formed by the difference in the mechanical and electrical phase behaviour (figs. 43, 44 and 45). We shall try to remove this conflict with the slip effect and by introducing a latent time.

2.1. *Slip effect*

De Vries c.s. (1952) already observed that at low frequencies the microphonic effect is ahead of the displacement and it was suggested that this might be due to the slip effect. If the slip occurs the hair will reach its moment of maximum stretching before the moment of maximum displacement. Already before the cupula is maximally displaced the hair creeps back to its initial position. The experimental results obtained now, for the whole frequency range (see figs. 43, 44 and 45), show a continuous change of the phase differences between the mechanical and electrical response of the organ.

At the higher frequencies the electrical response lags in phase behind the mechanical. It is not possible to make this plausible with the aid of the slip effect. But at the lower frequencies the slip effect gives a good explanation of the phase behaviour.

2.2. *Latent time*

In order to explain the behaviour at high frequencies we shall assume that the cell cannot react with a maximum response simultaneously with the maximum stimulus. Hence we now suppose that there is a constant latent time (τ) between the maximum stimulus and the maximum response.

The following example illustrates that this delay time will introduce a phase shift. If this latent time is e. g. 0,01 sec. then at a frequency of 50 cps. the microphonic effect lags in phase behind the displacement just half a period of the vibration. For a lower frequency, say 25 cps., a delay of 0,01 sec. corresponds with a quarter period, or 90^{o}.

The physiological arguments for the assumption of the latent time will be discussed in chapter XII.

In fig. 45 the dotted lines indicate the relation between the phase difference φ and the frequency in case τ is 1/0000, 1/800 and 1/600 sec. The line for τ = 1/800 sec (= 1,25 msec) fits the results best. In this figure we have assumed that the maximum effective stimulus to the cell is always 1/4 π ahead of the maximum deviation of the cupula. We arrived at this assumption by supposing that not the *elongation* of the hair but the *velocity of the elongation* is related to the microphonic effect. (see section 4. 1). This is not so important for the calculation of φ as will be shown below.

The latent time we calculated is rather large compared with the latent time found in the cochlea; there it is less than 0,3 msec. (Kahana et al. (1950)). It might be possible that the time we obtained is influenced by the operation of the organ (free cupula). To check this we calculated τ for a cupula in the natural situation from the change of the phase difference between the force and the microphonic effect, as given in fig. 45.

For this calculation we need the damping and the resonance frequency of the system (skin + fluid in the canal + cupula). The damping in this situation is approximately critical (see chapter V). Then the phase difference between the force and the mechanical response between ν_1 and ν_2 is $\frac{1}{2}\pi$, if both ν_1/ν_o and ν_o/ν_2 equal about 0,1 - 0,2 (see fig. 22-B curve 6).

At a frequency ν, the phase difference between the mechanical and electrical response of the cupula is $\beta - 2\pi\nu\tau$. In

this formula we have supposed that the effective stimulus to the cell is β^o ahead of the deviation of the cupula. In case we assume the velocity of the elongation to be effective, β is 45^o as will be shown in section 4. 1.

When in fig. 45 the frequency ranges from 10 - 100 cps. the observed change of the phase difference of the microphonic effect in respect to the force is about 140^o. So

$$140^o = 90^o + (\beta - 2\pi\, 10\tau) - (\beta - 2\pi\, 100\tau)$$

We see β is cancelled in the calculation of τ.

This gives τ = 1,5 msec. Between 100 and 500 cps. the phase of the microphonic effect changes over 300^o and this gives τ = 1,4 msec. The smallest value is obtained between 400 and 500 cps.; then τ = 0,8 msec. When the resonance frequency is not 100 but 50 or 150 cps. this does not influence τ very much.

So it seems plausible to assume a delay time in the origination of the microphonic effect of about 1 msec.

Now we have explained the change of the phase of the microphonic effect for the whole frequency range without using the slip effect. It is, however, certainly possible that a slip effect influences the value of β. In fig. 45 the dotted lines intersect the ordinate at 45^o. This means that at low frequencies there is a phase shift of 45^o. When these lines were constructed we ascribed this shift of 45^o to the velocity of the elongation of the hair but it might also be due to a slip effect.

3. Relation amplitude cupula - voltage microphonic effect

The relation between the current through the driving coil and the voltage of the microphonic effect is linear when the force is small (see fig. 40). At large amplitudes the input-output curve deviates from the linear course. This might be due to a "saturation effect" of the source of the effect. We shall leave this part of the input-output curve out of consideration here. As far as we know at present the amplitude of the cupula is directly proportional to the force applied. Therefore we take as an experimental fact: *the voltage of the microphonic effect is proportional to the amplitude of the cupula* ($V = k.a$). (The non-linear relation between a and x (see fig. 31) will be left out of account for the moment. A new model based on the considerations given here, is discussed in section 6.

From some measurements we got the idea that the *velocity* of the cupula is related to the microphonic effect. As a mea-

sure for the velocity we can use the amplitude multiplied by the frequency. So the statement $V = k.a.\nu$ is also checked.

3.1. *Results obtained from free cupula*

For the linear part of the curves such as given in fig. 41 and 42 we calculated the relation between a and V. The small amplitudes of the cupula are not measured but calculated from the relation between the amplitude of the force and the displacement of the cupula, as they were measured at higher amplitudes (see fig. 26).

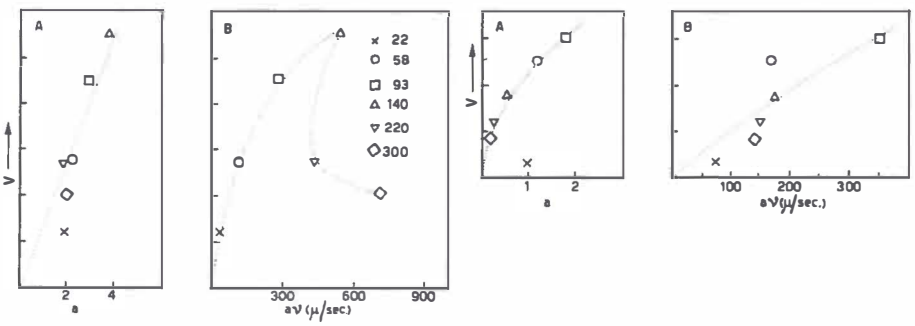


Figure 59. For two fishes the relation between amplitude of the cupula (a) and the voltage of the microphonic effect (V) in the figures A. In the figures B the relation between the velocity (a x) of the cupula and the voltage is given for the same measurements as used in figure A.

The results are given in figs. 59 and 60. There is a variability in the experimental results. De Vries, Jielof and Spoor (1952) observed no influence of the frequency. At higher frequencies than used by them, we now did not observe any influence of the frequency either. In other animals the frequency turned out to be essential for the microphonic effect.

It is not possible to give a plausible explanation of this variability at present. Physiological processes may have interfered. We will come back to this in chapter XII.

The best plan would be to study the relation between a and V at cupulae under the bone bridge (in the nearly natural situation). But the amplitudes are then so small, that they cannot be measured. It might be possible that at larger amplitudes which we obtained at the free cupulae not only the stiffness of the cupula is influenced but the sense cells as well. In connection with this it must be stated that at fish 81 (fig. 60) the amplitude of the cupula was relatively small; here the velocity was proportional to the voltage.

It is possible to get more information from the course of the frequency characteristic, however.

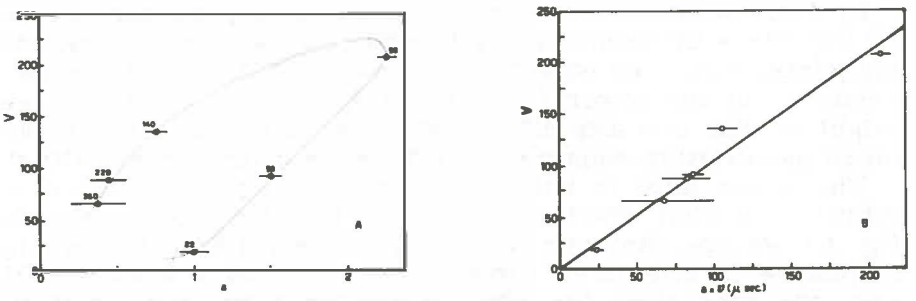


Figure 60. For fish 81 similar relations as in figure 59.

3.2. *Results from cupulae in the natural situation*

In the nearly natural situation we are dealing with a critically damped system (see chapter III). In this case there should be no maximum in the frequency characteristic, whereas we found that there is. With only the assumption $V = k.a$ we can give an explanation of the course at low frequencies with the aid of a slip effect. At low frequencies the hair slips so much that the effective amplitude of the cupula is less then the real amplitude of the cupula. At higher frequencies there is less time for the hair to slip and therefore, say in the resonance frequency (ca. 100 c.p.s.) a greater part of the amplitude is effective which results in a larger voltage of the microphonic effect. Above the resonance frequency the amplitude of the microphonic effect will then decrease about proportionally to the amplitude of the cupula. A more quantitative analysis will be given.

We usually found the maximum of the equal force curve at about 100 c.p.s. We take as a typical form of the equal force curve, number 3 of fig. 36. In fig. 61 we have normalised this equal force curve in such a manner that the maximum value of V is now 1.

Because we are dealing with a critically damped system we can now calculate the *course* of the relation between a and the frequency. This is given in curve 2. This curve would also give the course of the frequency characteristic when no slip (either mechanical or "cellular") occurs and the statement $V = k.a$ is correct. From these two curves we can calculate the relation between V and the frequency when the amplitude of the cupula is constant. In fig. 62 curve 1 the quotient V/a (voltage per unit amplitude) calculated from fig. 61 is plotted against the frequency. We call this the *equal amplitude curve*. If the idea $V = k.a$ was in agreement with the experimental results this equal amplitude curve should be a straight horizontal line.

Now the question arises whether we can explain the course of the curve by means of a slip effect as used in the preceding parts. Since the output decreases when the frequency decreases, at the lower frequencies the course of the curve might be due to a slip effect. We must, however, then make more quantitative enquiries into the time course of the effect.

The sense hair is being stretched during a quarter of a vibration period. During this period the slip must occur. In fig. 61 we see that at a frequency 25 only 20% of the cupula amplitude is effective. Consequently in 1/4 x 1/25 = 0,01 sec. the hair slips for 80%. In section 1 we found that the d.c. potential at constant displacements of the cupula was decreased to half of its initial value in about 0,1 sec. It will be evident that here we need a considerably faster process. Hence we now need a third component of the slip effect e.g. a very rapid one. It is more probable, however, that this is not a mechanical effect but a reaction of the cell.

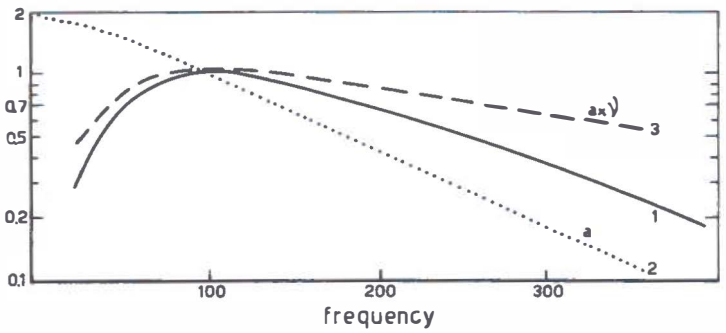


Figure 61. Curve 1 represent the normalised equal force curve 3 of figure
Curve 2 is the course of the relation between a and ν if the amplitude of the driving force is kept constant, in the case of critical damping and proper frequency of the resonator 100 cps. Curve 3 is the course of relation between a x and ν for the same presupposi;-tions as in 2.

We see in fig. 61 curve 1 that the effective amplitude of the cupula increases about proportionally with the frequency. Hence when the time becomes twice as short the amplitude is twice as effective. This suggests that a process is involved the influence of which decreases in proportion with the decrease of the time in which the hair is pulled.

It may be that 100 c.p.s. does not correspond to the mechanical resonance frequency but that a higher or lower one ought to be used. This does, however, not influence our conclusion that with the idea $V = k.a$ we need a rapid slip effect.

For the sake of completeness it must be remarked that at some fish the decrease of the voltage at the higher frequencies was not striking and in others the decrease at low fre-

quencies was not so pronounced. The animal we used above represents the average however.

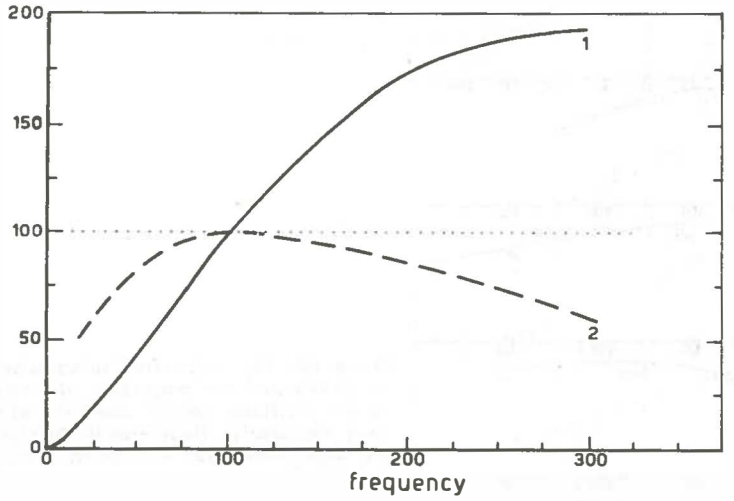


Figure 62. Curve 1 represents the course of the equal amplitude curve calculated from figure 61 the curves 1 and 2 and Curve 2 the equal velocity curve (see text).

When we state that not $V = k.a$ but $V = k.a.\nu$ we can calculate in a similar way the relation between V and the frequency at a constant value of $a.\nu$ Fig. 61 curve 2 gives the relation between V and the frequency at constant value of $a.\nu$. We call this curve the *equal velocity curve*.

If at low frequencies the equal amplitude curve was a straight line then the equal velocity curve must be horizontal. This is not the case. However, the equal amplitude curve was not entirely linear. Whether this deviation is due to a biological effect (any form of slip) or to inaccuracy of the measurements is unsolved at the moment.

For different resonance frequencies and the case of critical damping the course of the relation between the voltage and the microphonic effect was calculated, for the case $V = k.a$ and $V = k.a.\nu$. The curves are given in fig. 63. When we compare these theoretical curves with the experimentally obtained ones of fig. 36 and fig. 37 the agreement is again striking for the case $V = k.a\,\nu$. Important is that the presence of the maximum is a consequence of the assumption $V = k.a.\nu$. The difference in the place of the maximum can be accounted for by a difference in the proper frequency of the lateral line organ.

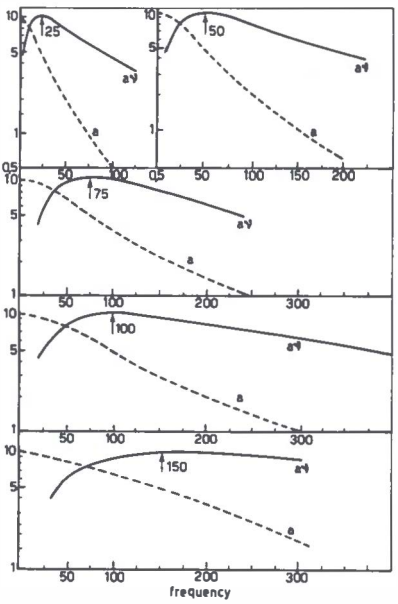


Figure 63. The calculated relation between the frequency and the amplitude of a critical harmonic oscillator (dotted line) at various resonance frequencies (indicates in the figures). The full drawn line gives the similar relation for the velocity (a x ν).

The results of this discussion concerning the influence of the velocity of the movement of the cupula suggest that the velocity is closer releated to the microphonic effect than the amplitude of the cupula. However in chapter XII we shall discuss that, whereas the amplitude of the cupula is significant for the stimulation of the cell, it is possible that the voltage of the microphonic effect is related to $a.\nu$.

4. Final discussion of the assumption V = k.x and the hair in perpendicular position

Our purpose was not to give a more or less adequate description of the electrophysiological results but to get an idea of the way in which the sense cell is stimulated. Therefore we will now turn to the model of the stretched hair.

4.1. *Velocity of the stretching of the hair*

In the preceding section we have seen that some difficulties can be overcome when we assume that the velocity is related to the microphonic effect. Or expressed in the terminology of the hair hypothesis not $V = k.x$ but $V = k.dx/dt$. So: the voltage of the microphonic effect is proportional to the velocity of the stretching of the hair.

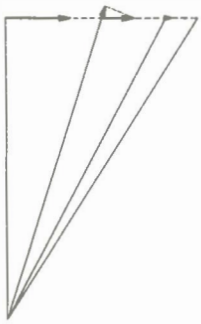


Figure 64. Diagram to show that the component of the velocity of the cupula in the direction of the hair is zero both when the cupula is in its equilibrium position, at half of the vibration amplitude and in the extreme position.

With this hypothesis some additional difficulties can be explained (see section 2. 2 of this chapter).

At the moment the cupula passes the equilibrium position, its velocity is maximum, but at this same moment the component of this velocity in the direction of the hair is zero (see fig. 64). At the moment the cupula has reached its extreme position, its velocity is zero, and consequently also the component in the direction of the hair. Between these two positions there is a moment at which the velocity of the change of length of the hair is maximum. This is 45° ahead of the maximum displacement. In agreement with this idea fig. 45 shows that at low frequencies the microphonic effect is about 45° ahead of the mechanical response of the cupula.

We used this model while calculating the course of the relation between frequency and the change in phase (fig 45). Therefore the dotted lines intersect the ordinate at $1/4\ \pi$

When a quick slip effect is used in this model ($V = k.dx/dt$), the microphonic effect becomes even more ahead.

We must amplify our supposition, dx/dt effective, by assuming that only the increase of the length of the hair is effective for the microphonic effect, because otherwise the electric effect would have a frequency four times the mechanical vibration.

A consequence of this extension of the hair hypothesis, so dx/dt is effective for dx/dt positive, is that the electric effect cannot have a true sinus form. The expected wave form of the potential is given in (fig. 65-d). Such an output curve (a sinus form with flat tops) is sometimes found (see also de Vries c. s. (1952)). In an experiment about the influence of the temperature (chapter X) they were very clearly visible (fig. 69). Under normal circumstances, however, the electric effect corresponds closely to the sinus form. An important advantage of the assumption $V = k.dx/dt$ is that with this idea we are able to explain the phase difference at low frequencies. But we have seen that with "slip" similar results can be obtained. Pending the discussion in the next section I want to state here that at the moment I do not believe in the idea $V = k.dx/dt$.

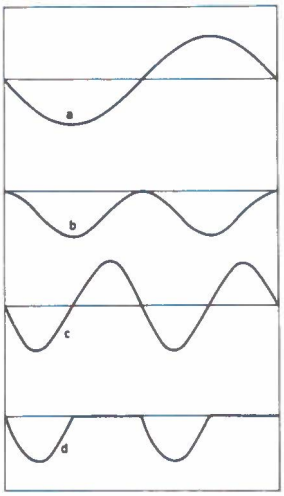


Figure 65. a. represents the movement of the cupula
b. indicates the change of the length of the hair x.
c. velocity of the stretching of the hair (dx/dt)
d. expected wave form supposing only the phase of the increase of the length of the hair is effective.

4.2. *Arguments against the idea $V = k.x$ if the hair is in the perpendicular position*

We have assumed that the hair on the sense cell is in the perpendicular position and that the microphonic effect is related to the extent to which the hair is stretched. Against this idea whether in the form $V = k.x$ or $V = k.dx/dt$ the same objection can be raised. At small amplitudes of the cupula we should expect in both cases $V = k.a^2$. So a quadratic relation between the amplitude of the cupula and the voltage of the microphonic effect, whereas, at small amplitudes of the cupula a linear relation is found.

We have tried to modify the hypothesis in such a way that a linear input-output curve is obtained. For example it might be possible that in the origin of the microphonic effect some cellular process is involved which acts in a square root manner. We have no stringent physiological objections against such a statement, because little is known on these cellular processes.

A biophysical discussion on the efficiency of the model shows, however, that the model hitherto used (hair perpendicular and $V = k.x$) is unlikely.

Essential to the hypothesis $V = k.x$ is that the component of the force in the direction of the hair is effective for the sense cells. In the lateral line organ as well as in the labyrinth organ, a shearing force is effective for the sense organ (see chapter V). This force acts approximately perpendicular to the direction of the hairs. According to the investigations into the mechanics of the cupula on the "nearly natural situa-

tion", the amplitude of the cupula is smaller than one micron (see chapter V). This means that the deviation of the hair, and consequently the component of the force in the direction of the hair, is very small and not more than 1/10 of the force available. Hence the mechanism is very ineffective.

De Vries a.o. (see his review 1956) has pointed out that the minimum theoretical threshold of a sense organ is determined by the thermal agitation in the sensorium. It is evident that the minimum "perceptible" energy applied to the sense cells will be of the same order of magnitude as that of the Brownian motion, because otherwise the sense cells would be continuously stimulated by the thermal agitation (see also chapter XIII).

In this respect the ineffective mechanism proposed in the hair hypothesis has consequences for the ear in particular. De Vries (see 1952 and 1956) assumed that the hairs are inclined to the direction of the effective movement and then using the most probable stiffness and coupling he could calculate that the energy applied to the cells was just enough to surpass the Brownian motion. But according to recent investigations of Wersäll the hairs are perpendicular. The length of the hair is about 4μ and a is of the order of $10^{-5}\mu$. Consequently such a minor fraction of the energy is applied to the sense cells that the signal-noise ratio becomes too small to be even probable.

On account of these energetic considerations the hair hypothesis, i.e. the notion that the microphonic effect is related to the extent to which the hairs are stretched, becomes unlikely (at least when the hairs are in the perpendicular position).

5. Two hairs, both inclined but in different directions

This extension of the hair hypothesis, like the slip effect proposed by de Vries, is that there are two types of hairs, one inclined to cranial, the other to caudal. Then one group of hairs is stretched during the half of a period of vibration and the other group during one other part of the vibration. On each cell only one hair is observed and therefore we must assume that there are two types of cells differing in the inclination of their hairs. It is true that there are no cytological observations to support such an inclination, rather the reverse (see chapter III). Yet we must pay attention to this idea because with this we are able to explain the linear course of the input-output curve. If the hairs are sufficiently inclined, the extent to which the hair is stretched (x) is directly

proportional to the displacement of the cupula (a) (see fig. 31). Hence according to the notion $V = k.x$, V increases directly proportional to a, as we observed.

Moreover the objections from the efficiency calculations (3. 3) can not be raised against this model.

We will elaborate this model (fig. 66) in connection with the slip. So we now assume that the hairs on the sense cells are inclined. To explain the course of the d. c. potential at a constant displacement of the cupula, we shall apply the slip effect to this model. When the vertical slip has occurred, like in the model with one hair (fig. 58) we have a functional situation as indicated in fig. 66-c. In this case both hairs are stretched by the vibration. This means that the microphonic effect due to the vibration, must have the double frequency when the d. c. potential had disappeared. We have to explain that it is single. To obtain from the model a single frequency we must then assume that the hair II (which becomes less inclined than it was in the resting cupula) needs more time to become stretched than hair I. Hence we assume that the vertical slip of the slack hair is slower than at the stretched hair. In the model of fig. 66 this seems indeed likely. There seems to be no reason for the slack "rope" II to creep back in the gelatine. This means that as long as the constant displacement is maintained the frequency of the microphonic effect remains single.

The decay of the d. c. potential after a constant displacement of the cupula might be a cellular reaction. On account of some physiological experiments (see chapter XII) this seems more likely. Anticipating these results there is no physiological argument against the idea that the hairs are inclined.

The present author prefers a different way to account for the linear input-output curve, which will be described in the next section. It must be emphasized here that our considerations in the next section also hold for the model of the inclined hairs.

6. The model of the gliding hair

From the investigations on the mechanics of the lateral line organ and the labyrinth organ we came to the conclusion that effective in these organs is the shearing movement of the gelatinous structure along the upper surface of the sense epithelium.

The hairs will now be considered as stiff rods which increase the friction between the two sliding surfaces, viz. between the lower side of the cupula and the upper surface of

the sense cells. By the sliding movement of the cupula a shearing force acts on the sense cells and these may be deformed as indicated in fig. 67. The size of the deformation depends on the stiffness of the upper part of the cell.

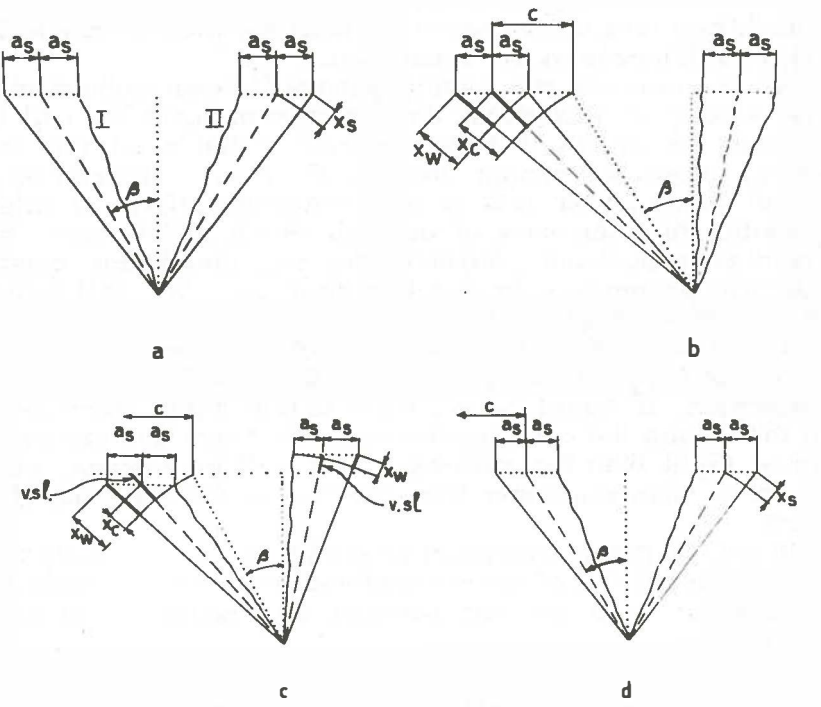


Figure 66. Similar figure as 58. but here the hair inclined . I indicates the hair stretched by the constant displacement C and v.sl. the vertical slip. Hair II becomes less inclined.

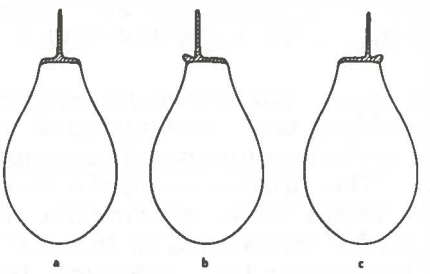


Figure 67. Diagram of the sense cell when the hair is considered as a stiff rod forming a coupling element between cupula and the upper surface of the cell.

There are two possibilities:
1. The voltage of the microphonic effect is related to the displacement of the hair. Because this displacement may be assumed to be equal or to be proportional to the amplitude of the cupula, we may state $V = k.a.$

2. The voltage is related to the velocity of the deformation. In this case the product amplitude cupula multiplied by the frequency is an indication for this velocity. Hence we suppose

$$V = k.a.\nu$$

This latter idea offers some difficulties when we try to imagine what happens at the sense cell.

At the moment the cupula passes its equilibrium position its velocity is maximum. At this very moment the cell is deformed not at all, but would produce the maximum microphonic effect according the idea $V = k.a.\nu$ Without an additional assumption this is not comprehensible. It might be possible that the part of the cell which is deformed, has no fixed zero position. Anyhow, the cell membrane must have specific properties to react in this way. We will return to this point in chapter XII.

Just like in the original model of de Vries the stiffness of coupling between the gelatine of the cupula and the hairs is important. It might be possible that at small displacements of the cupula the coupling between the hairs and the cupula is more rigid than the stiffness of the cell membrane, whereas at large displacements the contrary is the case and slip occurs.

In fig. 67 the deformation is exaggerated; in reality the deformation will be of macro-molecular order, possibly millimicrons or even less and perhaps only occurring at the base of the hair.

6.1. *The frequency doubling of the microphonic effect*

The sense cell is deformed twice each period of vibration and therefore the frequency doubling of the microphonic effect offers no difficulties.

In case the velocity of the motion is essential we might assume a similar additional hypothesis as described in section 4.1.

The microphonic effect of the cochlea has indeed the single frequency and this offers a new problem now. Compared with the lateral line organ the cochlea is from an anatomical point of view a highly developed organ. The difference in the frequency of the microphonic effect might be a consequence of this development. It may be due to the mechanics of this organ or to specific properties of the sense cells. Whether the microphonic effect of the semicircular canals has the single or the double frequency is not established at the moment. The sacculus produced the double frequency (see de Vries (1952)).

6.2. *The linear input-output curve*

Since as far as we know the amplitude of the cupula is directly proportional to the force applied we expect a linear input-output curve.

The considerations given in section 3.2 about the frequency characteristic can be applied to the new model.

6.3. *Phase behaviour*

In case the amplitude is significant to the microphonic effect we can use an explanation similar to that given in section 2. With a kind of rapid slip here too, at low frequencies, the microphonic effect becomes in phase ahead of the displacement.

In case the velocity is essential the microphonic effect ought to be 90^{o} ahead of the amplitude of the cupula. We observed 45^{o}, but in 1952 greater values were observed. When slip occurs the discrepancy between experimental results and the theory are even more increased. This question is not solved and needs further attention.

The course at the higher frequencies can be described with the latent time as before (section 2.2).

6.4. *The electrical behaviour during constant displacement of the cupula*

Previously we have explained this with the slip effect. Now this offers some difficulties. A vertical slip effect does not occur in the new model and therefore we ought to modify the explanation of the difference in the time course of the d.c. potential and the doubling of the frequency after constant displacement.

In case $V = k.a$ the time course of the redoubling of the frequency is sufficiently accounted for, if we assume that the slip effect is a slow process. Then an other explanation must be given for the decay of the d.c. potential. It might be due to the sense cell.

In case the velocity is essential ($V = k.a_{\nu}$) we cannot explain why the frequency becomes single after a constant displacement.

6.5. *Superposition effect*

This is inexplicable by means of the model we assumed in this section, because the model is of a linear character, whereas the superposition effect is a non-linear reaction. We have to look for another explanation of the effect. It might be due to the sense cells, see chapter XIII.

7. Conclusion

Summarising the results of the considerations of this chapter we may say:

1. The slip effect has proved to be a useful assumption to explain the course of the microphonic effect during constant displacements of the cupula.
2. The phase behaviour at low frequencies can be explained with a slip effect, at any rate if the amplitude of the cupula is significant for the microphonic effect.
3. The phase behaviour of the microphonic effect at higher frequencies can be partly explained with a latent time.
4. The hair hypothesis in the formulation "the hairs perpendicular to the direction of the motion of the cupula and $V = k.x$" is disproved.
5. When two inclined hairs are assumed various experimental results can be explained. But on account of the histological observations this idea seems less likely.
6. At the moment we prefer the idea that the hairs on the sense epithelium are displaced as a whole. They increase the friction between the two sliding surfaces i.e. between the lower part of the cupula and the upper surface of the sense epithelium. With this model we can explain the linear part of the input-output curve at a given frequency.

There are some indications that the voltage of the microphonic effect is related to the velocity of the cupula, so $V = k.a$. (see section 3). However, at the moment it seems more probable to me that for the cell the displacement of the cupula is essential, and that cellular processes are involved in the origin of the microphonic effect by which $V = k.a$. shows a better agreement with the experimental results than $V = k.a$.

7. The superposition effect is inexplicable with the models hitherto used.

The general conclusion must be that with a "merely physical model" we are unable to give a complete description of the experimental results we have obtained. We have to bring into the discussion processes at the sense cell itself.

The experiments described in the preceding chapter were designed on the basis of the mechanics of the hair. We shall now abandon this way of approaching the problem and turn our attention to the physiological processes in the sense cell. Hence we will discuss the source of the microphonic effect and try to design our experiments according the second hypothesis of de Vries (chapter VI).

Chapter IX

INTRODUCTION TO A MORE PHYSIOLOGICAL APPROACH TO THE MICROPHONIC EFFECT

So far we had considered the variable resistance localised in the top of the sense cell (in figure 29), more or less as a kind of rheostat the sliding contact of which moves as a result of the pulling on the hair; hence we assumed the voltage of the microphonic effect to be proportional to the amount to which the hairs are pulled. This idea has proved to be an oversimplification.

With the cell we are dealing with a membrane potential – a potential difference between the inner and the outer side of the cell. Such potentials are related to a difference in the concentration of ions on either side of the membrane. The cell membrane is likely to consist of complex compounds of proteins and lipoids. In the resting situation it is practically impermeable to anions and certain kations but becomes permeable when the cell is stimulated.

The same will be the case with the sense cells of the lateral line organ. The top of these sense cells must be particularly sensitive to mechanical forces. The histological investigations have indeed demonstrated a structural difference between the apical and basal part of the cell (see chapter III).

Our problem in the initial stage refers to the change in permeability of the cell membrane, In this connection it is of importance to know what other investigators have accomplished regarding similar processes in other biological structures. A survey of the literature is therefore given in the next section.

1. Survey of the literature on membrane potentials

Permeability changes are of central interest in physiological literature at the moment, since they are connected with fundamental problems in biology, viz. the transport of metanolic substances, hormones, ions, etc. to and from the cell. Both from physical and chemical quarters there is a formiable quantity of literature describing models in which similar processes take place; the biologists on the other hand are also concerned with such processes in living material. Though the ideas of the workers on both fields are approaching each

other more and more, there is still a great deal of discrepancy between them. The physico-chemical models are too simple and do not give a satisfactory explanation of the biological process. The experimental facts collected by the biologist, on the other hand, are so complex that the physico-chemist has no notion of the underlying mechanism.

However, investigations on membrane potentials of nerves, muscles and electric-organs have shown that in principle we are dealing with the same mechanism in these organs. I shall give a short survey of the literature on the elementary mechanism of nerve- and muscle-potentials, as a similar mechanism may be expected for the sense cells. To avoid a long discussion I left out the methods and the names of the various investigators. For extensive reviews see Hodgkin 1951. Wyke 1951.

We must distinguish two cases: a) *resting potential*, which is the membrane potential of a quiescent cell.

b) *action potential*, which refers to the potential of a membrane along which a disturbance is travelling. The former informs us about the battery in the cell and the second about the change in resistance of the cell during activity.

1.1. *resting potential*

The individual cell may be considered as a small electric battery, in the sense that it possesses an electromotoric force which maintains a potential of about 50-100 mV – over the interface between cytoplasm and the external environment. The outer side is positive with respect to the inner side. The membrane is about 50 Å thick and has the properties of a rectifier. It behaves as a condensor with capacity 1 μ F/cm^2, parallel with a variable resistor, resting value 1-2000 ohm/cm^2 and a battery. The potential difference depends on the concentration of ions at the outer- and inner side of the cell.

The first approach to the origin of the resting potential has been the assumption that we are here concerned with a Donnan equilibrium. A filtering activity was ascribed to the cell membrane and unlike the smaller cathions, the larger anions were supposed to be unable to diffuse through the membrane.

Recent investigations with tracers have shown that various anions which were originally supposed to be incapable of passing the sieve can do so; this holds good for a. o. inorganic phosphate lactate. For the rest this theory does not account for the fact that sodium may pass the membrane in both directions, though in resting position its concentration is 3-15 times larger outside the cell than inside. The same

has been found for potassium, which is 20-50 times more concentrated on the inner side.

So the membrane or sievetheory in which ionic accumulation was regarded as a passive result of differential ionic permeability of the membrane caused by difference in size of ions and the size of the pores- is not supported by modern tracer-experiments. This theory must be considered an oversimplification giving no complete description of the biological processes.

Recently Ling (1951, '54) proposed another system. He abandons the cell membrane as a functional unit for accumulation of ions and regards this accumulation as a physicochemical consequence of the structure of the cytoplasm itself. The cell-protein contains a great number of fixed anions. The Na^+ and K^+ tend to approach by coulomb forces as closely as their respective diameters will allow. Because the hydratation "coat" of sodium is considerably larger than of potassium, the fixed anion K^+ combination represents a lower energy state. The whole system tries to reach the lowest energy state and therefore the cells will take up the smaller K- and not the bigger Na^- ions. The theory is called the fixed charger theory.

In another theory the resting potential is explained by assuming an active extrusion of sodium by the cell, so that the distribution of potassium and chloride, which can both freely diffuse through the membrane, is identical to a distribution we obtain when the membrane is interpermeable to sodium.

At present this conception of the resting potential is the most usual one for muscle and nerve. It is supported by the above-mentioned permeation of the three important ions Na^+, K^+. Cl^-, discovered with tracers, and the extrusion system, especially demonstrated in erythrocytes, frogskin and kidney cells. Moreover the resting potential is of the size to be expected from calculations for a membrane impermeable to Na. Further it appeared that the resting potential was dependent on the concentration of potassium (Hodgkin 1951).

The question arises, whether the extrusion of Na^+ is caused by an active process of the cell itself. Such a pumpsystem needs energy and this will be supplied by metabolic processes. Under normal conditions Na- concentration inside muscle or nerve cell does not vary much so that in this situation there is an equilibrium between the inside and the outside flux of sodium. The flow in the second direction is energetically expensive, for it runs counter to both the concentration and the electric gradient. The gradients must be measured and the number of Na-ions extruded per time and square unit determined with the aid of ^{24}Na. The minimum rate of energy expenditure of the pump is thus obtained. The energy con-

sumption of the cell during this situation can be measured as well. Ling 1951 calculated for frog muscles that the minimum energy the sodium pump needs exceeds the maximum available energy. In other cases it has been calculated that the pump used 20-40% of metabolic energy. For the time being there are not enough data to draw any definite conclusions.

Nearly all workers in this field are in agreement that the resting potential cannot be maintained over a long period in the absence of metabolism. Regarding the manner in which metabolism is connected with the resting potential and the ionic concentration difference there are many theoretical suppositions but far less useful experimental facts.

Pharmacologically it has been demonstrated that after blocking of the metabolism (with cyanide) the resting potential will be maintained for several hours after complete elimination of the metabolic apparatus.

For the pump Ussing (1952) proposed in the membrane a carrier system which ferries Na^+ back and forth between the inner- and outerside. In this way it is possible to evade the energy problem partly. A combination of a carrier mechanism and the fixed charge theory is of course also possible.

It is not possible at the moment to gain a clear idea of the mechanism of the membrane potentials in living cells. The details of the mechanism will probably remain unsettled for some time. For the present discussion it is sufficient to know that the membrane potentials are related to the difference in ionic concentrations at the inner and outerside of the cell. The height of potential approximately equals the potassium concentration potentials.

1.2. *Processes during activity*

Both for nerve and muscle there is experimental evidence that during activity i.e. if an impulse runs along the fibre there is an inflow of sodium and an outflow of potassium. This ionic current is the result of a change in the membrane conductance for sodium and potassium.

Electro-physiologically the giant axon of the squid is the best studied object so far. In beautiful experiments Hodgkin and Huxley 1952 have shown that the conductances both for Na^+ and K^+ undergo a characteristic but different increase when the membrane potential difference is reduced to zero. For the sodium ions the conductance rises in 1-2 msec. from 0.01 mmho/cm^2 to about 15 mmho/cm^2 and then drops to a low value with a time constant of about 1 msec. That for potassium rises along an S-shaped curve, and remains at about 20 mmho/cm^2 as long as the membrane potential difference is kept at zero. On restoring the membrane potential difference to its ordinary resting value the K-conductance returns

exponentially to its resting value (0,5 mmho/cm^2). These different permeability changes can be found again in the action potential of the nerve. The rising phase is caused by the sodium inflow which may lead to a reversal of the membrane potential. The falling phase is slower; this is the phase of decrease of Na^+ permeability and the increase of the K^+ permeability. The K-ions move outwards in the direction of their concentration gradient just as Na^+ inwards. In this way the fibre is brought back to its resting potential (50 mV). When this value is reached, there is still a K-current outward.

These experimental results prove that the potential difference across the membrane arranges the permeability. We may then think of charged particles in the membrane, which move under the influence of an electric field or influence a chemical reaction by which the permeability changes, e.g. because the openings in the membrane are enlarged. The steepness with which the conductivity changes suggests that the relevant particle is multiply charged or else that several singly charged particles are involved at each side where ions can penetrate.

For the permeability controlling mechanism there is on the other hand experimental evidence e.g. from the work of Nachmansohn and his coworkers showing that acetylcholine (ACh) is responsible for the generation of the action potentials. It has been proved that at least in a number of cases ACh is responsible for the permeability change. In the electric organ of Torpedo, injected ACh generates a change of the membrane potential.

It is not certain that ACh is involved in the origin of action potentials in nerve fibres. Different investigations have made it very probable that in nerve muscles connection ACh is liberated and that this substance is responsible for the rise of a local potential at this connection. When this local potential reaches a critical value it initiates the muscle action spike.

The precise action of ACh is still obscure. There are some data suggesting that it may change the configuration of protein. It is probable that ACh in resting condition is inactive, for it is bound to a protein or lipoprotein. Experimentally it has been extablished that it is free during activity; How it is released is not known. The inactivation and synthesis of the substance and its relation to metabolic processes are fairly well established (For ref. see Nachmansohn).

2. Application to the sense cells of the lateral line organ

With the above brief discussion in mind, we must try to

arrive at a more physiological approach of the microphonic effect, i.e. to the processes induced in the sense cell by the deformation of the cell.

In the first place it is fairly probable that in the sense cell a similar mechanism is responsible for the E. M. F. as is found in nerves and other excitable tissues. To give experimental evidence of this will be the aim of future experiments. The mechanism will be different from that in nerves for there the current flows for about 1 msec and this time is much longer in the lateral line organ. The electric effect of the labyrinth organs and of the lateral line organ shows more resemblance with the local potentials, for example the potential at the nerve muscle connection. In the origin of these potentials ACh is known to play an important part. Therefore it seems possible that as a consequence of a deformation of the membrane at the top of the sense cell, a conductivity increasing substance like ACh is liberated.

The increasing of the permeability by ACh must be counteracted by a reaction of the cell inactivating ACh.

The introduction of a double system, a conductivity increasing (production of ACh) and a conductivity decreasing mechanism (inactivation of ACh), strongly increases the number of theoretical possibilities. Then the electric effect depends on the difference between the two mechanisms.

Experimental indications of the presence of such an ACh-mechanism will be given in chapter XI.

Secondly the results of the investigations into the alternations of the direct-current-potential will be given in chapter XII. Although these experiments are originally based on the hair-hypothesis, the results have more connection with the processes at the cell than was the case with the preceding experiments.

In chapter X some miscellaneous experiments are described. They are given to demonstrate that the similar effects described for the microphonics of the labyrinth organs are also obtained at the lateral line organ.

There are various aspects which will be left out of consideration at the moment, because the experimental result I have hitherto obtained are too fragmentary to warrant definite conclusions. This is the case with the influence of ions, sodium, potassium, magnesium and calcium and with substances affecting the metabolic processes in the cell.

Chapter X

SOME GENERAL PHYSIOLOGICAL ASPECTS OF THE MICROPHONIC EFFECT

From the discussion of the literature on the microphonic effect of the labyrinth organs (see chapter IV) we have seen that the microphonic effect depends on the physiological condition of the animal. In this chapter it will be shown that the same applies to the microphonics of the lateral line organ.

1) Condition of the animal

The microphonic effect is only found in living animals and not in dead (here used in the ordinary sense of the word). The frequency characteristics given in chapter VIII figures 36, 37, and 39 show a considerable difference. It is clear that the variation between different animals cannot be neglected, It is however remarkable that in one animal different cupulae have a similar frequency characteristic (fig. 36-C, 3).

In this connection it seems of importance that sometimes the macula was not the normal yellow colour, but a pale, often a white colour. At these cupulae the microphonic effect is always small or unmeasurable.

2) Importance of bloodsupply

When the artery leading to the cupula is cut, the electric effect decreases. This cutting causes severe hemorrhage which stops after some minutes. The animals survive this operation very well. After such an experiment they behave quite normally. The effect of the cutting on the electric activity is given in fig. 68. During the first few hours the microphonic effect remains constant, then it decreases in about 2 hours to nearly 20 percent of the original value. Consequently the energy present in the macula is sufficient to maintain the constant output during the first few hours. Whether or not the decrease occurs sooner when the cupula is stimulated has not been studied in detail. I have not the impression that there is a great difference.

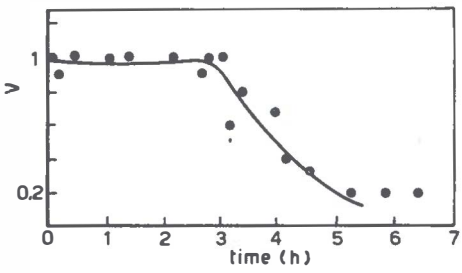


Figure 68. The decrease of the voltage of the microphonic effect, at a constant input, after stopping of the bloodsupply to the cupula at time "zero".

3) Influence of the temperature

In a former investigation no effect of the temperature was observed on the electric effect of the lateral line organ (see de Vries et al. 1952). I have studied the influence of the temperature once more, but this time with a better apparatus than the former investigators (Jielof and Spoor) had at their disposal. It appeared that there was an effect both on the amplitude and on the form of the potential.

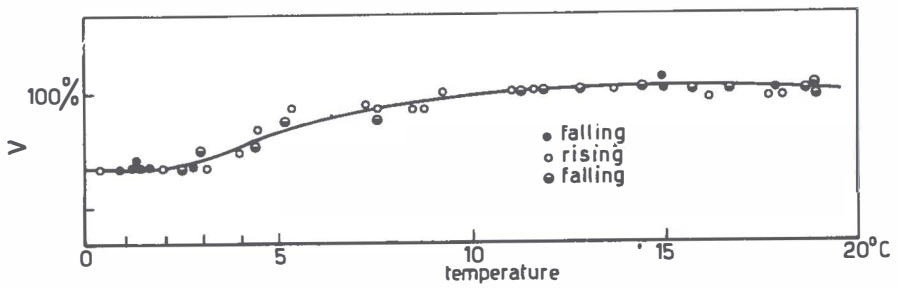


Figure 69. The interrelation of the temperature of the water in the fish tank and the voltage of the microphonic effect at a constant stimulous. The temperature was first dropped from 15° to 0,5° C., then raised to 18° and thereupon to 2° C. again.

With lumps of ice the temperature of the water was lowered. It was measured by means of a small thermometer near the head and at the height of the cupula. Before each measurement the water near the head was stirred with a glass bar. The place of and the current through the driving coil were kept constant. The results obtained from one cupula are given in fig. 69.

From 20° to about 10°C, there is no noticeable effect of the temperature, from 10° to 3°C. the voltage decreases with about 50 percent. Below 3° C. there is no further effect of the temperature. The effect is completely reversible, i.e. the

relation between the temperature and voltage of the microphonic effect is the same for the descending and the ascending temperature.

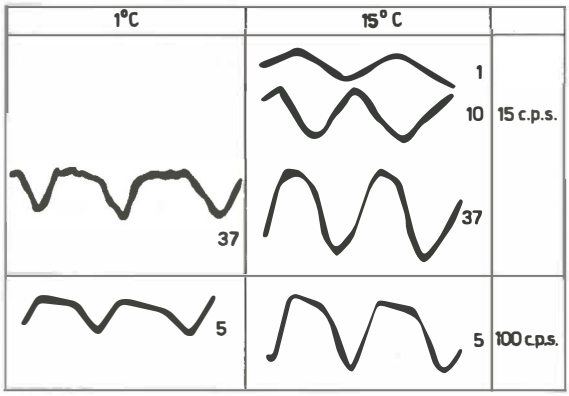


Figure 70. The waveform of the microphonic potential at 1 and 15° C., both at 15 and 100 c.p.s. Near the curves the input values are indicated.

Figure 70 shows the course of the potential for the frequencies 15 and 100, both at about 1°C. and 15°C. This shows that the form of the potential is also influenced by the temperature. At 14 c. p. s. and 1°C. flat tops appear which are not visible at the same electrical amplitude at 15°C. At 100 c. p. s. the phenomenon is less pronounced. A change of the frequency characteristic could not be clearly demonstrated.

4) Chemical influences

As a narcotic for the fish aethylurethan was always used in solutions of 1/2 to 1 per cent in the respiration water. No influence of this on the electric effect could ever be demonstrated. This is in agreement to what was found for the electric activity of the cochlea and the semicircular canals (Bleeker). Alcohol and ether diminish the effect. Methylene blue, often used as a vital staining substance, also in labyrinth physiology (Békésy), has a fatal influence. Some seconds after the introduction of the dilution into the canal, the microphonic effect had disappeared. As soon as there is a slight colouring of the macula no electric effect is detectable. This makes it probable that oxydation processes play an essential part in this phenomenon. I have studied the influence of flaxedil, a substance with a similar pharmacologic effect as curare, more extensively. Under the influence of this substance the effect disappears reversibly. We shall return to this in chapter XI.

The influence of ions has not yet been examined in detail. Preliminary measurements point to a decrease of the effect by potassium whereas sodium has little effect. Magnesium chloride causes a marked, reversible decrease of the potential. This is probably based on a similar influence on the generator system as found with flaxedil.

5) Conclusion

When comparing the electric effect of the lateral line organ and the similar effect of the labyrinth organ (see chapter IV) from a general physiological point of view we observe an agreement. In both organs the effect depends on metabolic processes, as is indicated by the importance of the blood-supply, metabolic poisons and the effect of the temperature.

On the other hand, the preliminary observations of the effect of ions lead us to surmise that the processes involved in the origin of the microphonic effect are similar to those found in the electric effects of nerve, muscle, etc.

Chapter XI

THE EFFECT OF A CURARE-LIKE SUBSTANCE ON THE MICROPHONIC EFFECT

This chapter describes some experiments with which I have attempted to find out whether there is a chemical intermediate between the pulling on the hair of the sense cells and the microphonic effect.

Acetylcholine (ACh) is one of the substances to be considered. At the nerve muscle connection ACh is liberated by the nerve impulse. In this case the effect of ACh can be blocked by curare and related substances. Curare seems to settle on the membrane receptors for Ach and thus prevents the action of the liberated ACh.

Katz and del Castillo and Engbeak and del Castillo have proved that magnesium has a similar effect as curare. This may act on the enzyme system for the production of ACh.

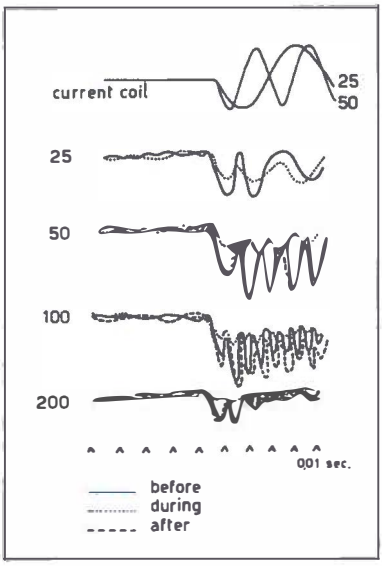
If in the lateral line organ ACh has a function in the chain of reactions resulting in the electric activity, a curare-like substance like flaxedil should decrease the electric activity of the organ.

1. Methods

In these experiments a dc-oscilloscope was used (see chapter II). The fish used were partly narcotised. In the flaxedil experiments the pharmacon was dissolved in ordinary tap water and brought into the canal of the lateral line organ with the aid of a pipette. The driving was magnetic with a magnet mounted on the skin. It was checked that the flaxedil had no effect on the conductivity of the water. The test solution was removed by sucking the fluid away with a small pipette.

2. Results

In figure 71 the electric effect is shown for four frequencies, before, during, and after application of flaxedil. In these experiments the pharmacon was dissolved in ordinary tap water and brought into the canal of the lateral line organ with the cation of flaxedil and finally after removal of flaxedil when the output had reached its normal value. In all the experiments the top values of the currents through the driving coil were the same (indicated in the upper figure). There were no indications that the "żero-line" had moved after flaxedil ap-



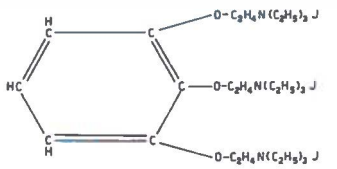


Figure 71. The change of the voltage at the onset of the vibration, before, during and after the flaxedil application. The d.c.-potential at the resting cupula was used as reference line when the recordings were projected over each other.

plication and hence it was used as a reference line during the projection of the photographs over each other. The typical way of recording the microphonic effect was chosen so as to be able to study also the course of the voltage curve at the starting of the vibration.

Flaxedil decreases the microphonic effect considerably, but after removal of the solution the effect returns to its original value. Because the sensitivity to flaxedil is less at low frequencies, the system becomes relatively more sensitive to the lower frequencies. The change of the frequency characteristic might be caused by an influence of the pharmacon on the mechanical system. This is not very probable because there is in either circumstances a similar transient effect. The frequency of these transient oscillations is about 150 c.p.s., which corresponds to twice the mechanical resonance frequency (about 75 c.p.s.).

The effect of flaxedil on the input-output curve has also been studied. Figure 72 shows the results.

First the amplitude of the electrical effect at 300 c.p.s. was decreased with flaxedil to about 75% of the initial value (fig. 72-F). In this situation the input-output curves at lower frequencies were studied. The lower the frequency the smaller was the effect; at 15 and 25 there was hardly any effect and at 50 c.p.s. only at higher amplitudes. After removal of the pharmacon the cupula was electrically more sensitive to 50 c.p.s. than before the application. This was not the case at 200 c.p.s. In a second experiment the output at 200 c.p.s.

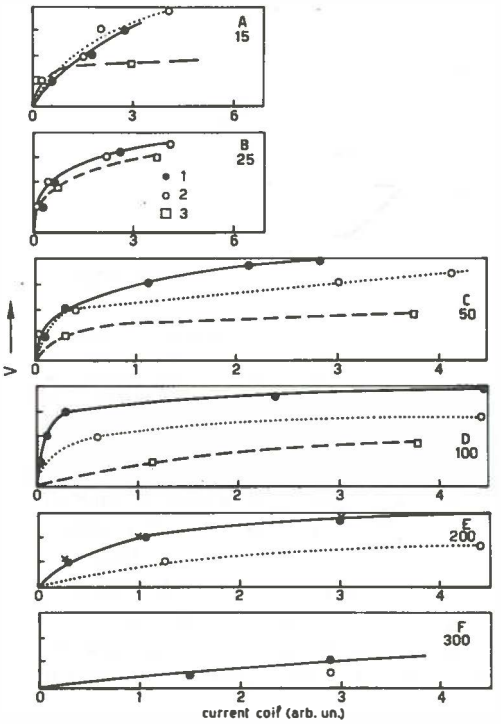


Figure 72. Input-output curves at different flaxedil concentrations. The frequency is indicated under the letter of the figure.
The full drawn line (curve 1): normal conditions. Dotted line (curve 2), the voltage at 300 c.p.s. dropped with about 25% by flaxedil. Dashed line (curve 3) at 200 c.p.s. the voltage dropped about 50%.

was decreased with 50% by means of flaxedil. In this case, too, no marked effect of flaxedil at the small amplitudes of the low frequencies could be noticed.

On account of the measurements described in the previous paragraph we may state that the sensitivity of the lateral line organ to flaxedil depends on both the amplitude and the frequency of the microphonic effect. The dependence on the frequency is also demonstrated in the equal response curve in figure 73. In this case the voltage of the microphonic effect was 50 μV, so for all the frequencies we worked in the linear part of the input-output curve.

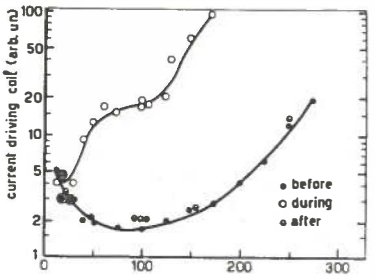


Figure 73. Equal response curves of the microphonic effect before, during and after the application of flaxedil.

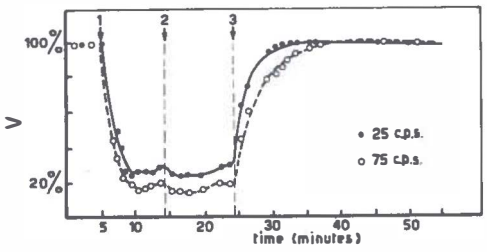


Figure 74. Course of the amplitude of the microphonic effect at a constant input, when flaxedil is applied. At 1 and 2 the flaxedil solution is applied and at 3 it is replaced by tapwater.

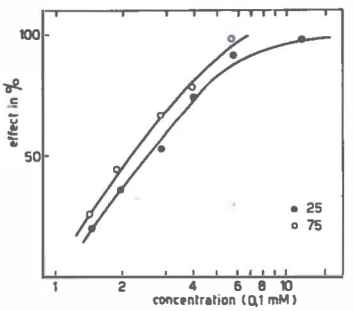


Figure 75. The dosage response curve of flaxedil for the microphonic effect of the lateral line organ. The relation is given for two frequencies.

The timecourse of the inhibitory action of flaxedil is of importance; this is given in fig. 74. The dosage-response curve (of figure 75) for flaxedil (after 5 min. application) was obtained with the aid of such like figures. After the removal of the pharmacon the potential sometimes climbed to a higher value than it possessed before the application of flaxedil. Whether this was caused by a better driving situation or by a real effect of the flaxedil treatment, remained uncertain.

3. Discussion

The results of the flaxedil experiments suggest that an ACh-mechanism is involved in the generation of the electric activity in the lateral line organ. To the best of my knowledge this is the first time it is demonstrated that acetylcholine is active in sense cells.

Of special importance is the question whether we are dealing with a specific reaction of flaxedil with a distinct receptor, i.e. a molecule to molecule reaction. It is also possible that we have to do with a toxic effect here. The concentration necessary to obtain a diminution of the electric activity of 50% is rather high (0,3 mM) as compared with a similar effect in the motoric endplate in coldblooded animals. In the frog about 0,5 mM is sufficient to give a complete paralysis.

The reversibility of the reaction is very good and at different animals similar results were obtained. These facts suggest a specific action effect. Experiments with $MgCl_2$, which will be discussed in detail somewhere else, give the impression of a mechanism similar to that in the nerve-muscle connection.

The sensitivity to flaxedil depends on the frequency of the

oscillation. Especially at low frequencies and at small amplitudes flaxedil has no effect on the voltage of the microphonic effect.

We may describe the results obtained with the general statement that flaxedil causes a retardation in the electrical response of the lateral line organ. Then the higher frequencies are affected especially. The consequencies of this way of describing the effect will be checked in future experiments.

In the superposition experiments the sensitivity to high frequencies is increased by the presence of a low frequency. This might be caused by the mechanism which is influenced by flaxedil. But, worse luck, so far I have failed to prove this. In these experiments the amplification of the untreated organ was rather small (A = circ. 3), and this small amplification made it technically difficult to measure accurately a relative decrease of the amplification. These experiments will be repeated in the near future.

If just as in the nerve-muscle junction the action of the curare-like substances is a surface phenomenon and the amplification is an intrinsic effect of the cell interior we should not expect any influence of flaxedil on the amplification. At the moment I cannot form a definite conclusion.

It seems worthwile to study the effect of curare-like substances by local application to the labyrinths of warmblooded animals. This type of experiment is in preparation now. Anaesthetic concentrations of curarine injected into bloodvessels seem to have no effect on the microphonic activity (a. o. van Eyk).

Chapter XII

DIRECT CURRENT COMPONENTS OF THE MICROPHONIC EFFECT

In the descriptions of the experiments given here, we shall proceed upon the following assumption: Pulling of the hair on top of the sense cell increases the conductivity for special ions in this part of the cell. Hence there is a current around the sense cells and this is observed in the form of the microphonic effect.

The experiments with flaxedil discussed in the foregoing chapter, render it probable that acetylcholine is involved in this change of conductivity. The question now arises whether the membrane resistance recovers its original value after each half of the vibration period. It may be possible e. g. that not all the ACh produced after the period in which the hair is pulled is inactivated. In this case the membrane resistance will not be completely restored and there will remain a direct current, apart from the alternating current hitherto considered. The degree in which this component changes with the amplitude and the frequency of the vibration will inform us on the recovery mechanism. The direct current component of the microphonic effect during constant displacement of the cupula was already described in chapter VII.

1. Methods

The techniques of these investigations were described in chapter II. A dc-oscilloscope with a slow horizontal time-sweep was used so that the separate oscillations cannot be seen in the recordings and consequently only the "enveloping" figure is visible (fig. 76). One sweep from the right to the left was photographed and during a certain period of this time the current through the coil was closed; hence the cupula vibrated during this period.

2. Results

The pictures of fig. 77 are reproductions of oscillograms of microphonic effects during which the cupula is first at rest, next vibrates and finally is at rest again. Fig. 76 gives a schematic representation. In this figure 1 indicates the

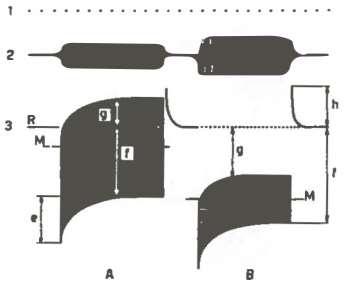


Figure 76. Diagram of the various components of the microphonic effect. 1 time marks; 2 current through the driving coil; 3 microphonic effect; R dc-potential of the resting cupula; M mean value of the oscillating potential of the vibrating cupula; e on-effect; f the amount the negative tops are below R; g the amount the positive tops rise or fall below R; h the off-effect. In B both amplitude and frequency of the input are increased in respect to A.

time, 2 gives both the period in which the cupula is vibrating and the amplitude of the force applied; 3 represents the electric response of the cupula. The alternating current described in the foregoing parts is indicated with (*f+g*): it equals *V*. For (*g*) both a positive (fig. 76 A-3) and a negative value (fig. 76 B-3) are found, depending on the amplitude and the frequency of the driving vibration. By (*e*) and (*h*) an on-effect and an off-effect respectively are indicated.

We have already seen that at the resting cupula there is a current round this organ; this means that the resistance at the top of the cell (fig. 29) is not infinite. The potential observed at the resting cupula is indicated by *R* and the mean of *V* (the ac-component of the microphonic affect) by *M*.

These various dc-components will be discussed separately.

2.1. *The on-effect*

The height of the on-effect (*e*) depends on the amplitude of the vibration applied, as can be seen in the recording of fig. 77.

The course of the decrease of this effect is of particular interest. Therefore in fig. 78 the magnitude of the on-effect is plotted (logarithmic scale) against the time (linear scale). It appeared that the on-effect is not a simple exponential process. The second part of the process is exponential. In the figure the line for this is extended to the left (dotted line). The difference between this part and the experimental curve is plotted in the same figure (dashed line). It appeared that the on-effect could be represented very well as a sum of two exponential processes, except at low frequencies in which only the fast process is present.

2.2. Direct potential when the cupula has vibrated for some time

The oscillograms of fig. 77 show that *g* can be both positive and negative. In the first case the membrane resistance reaches each period a higher value than in the resting position, in the latter case it does not reach this position. The

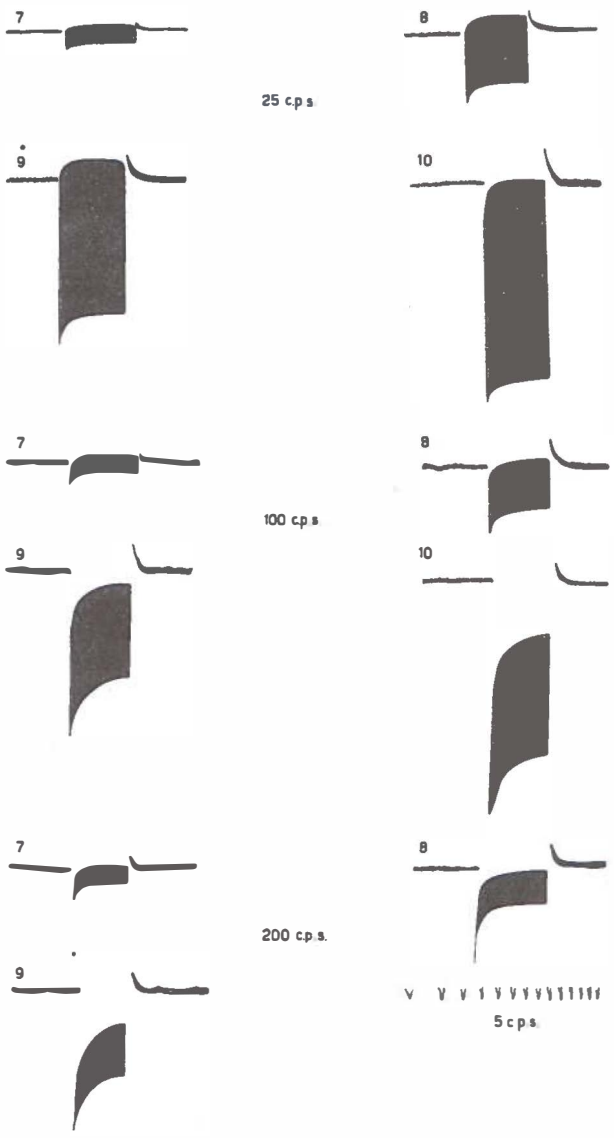


Figure 77. Reproduction of oscillograms to demonstrate the dc-components of the microphonic effect (see fig.76). The cupula vibrates only during a part of the recording. The separate oscillations are invisible due to the slow sweeprate. The time scale indicated is the same for all the recordings. The arbitrary values of the input are attached to the recordings.

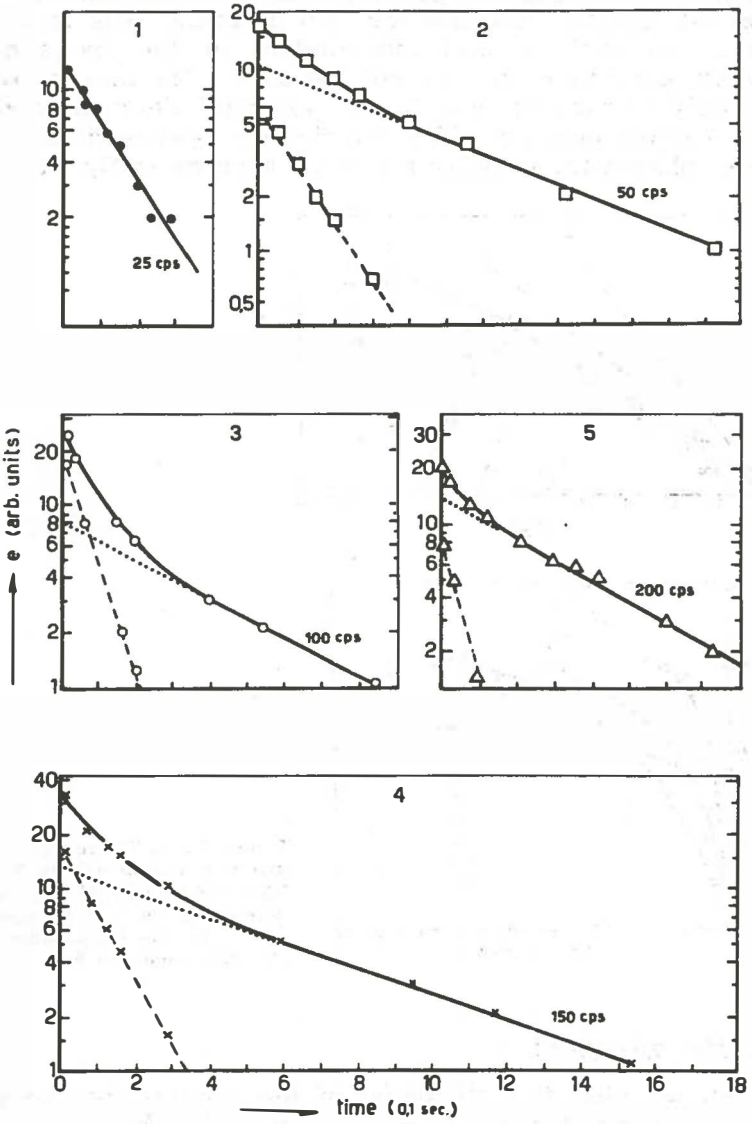


Figure 78. The time-course of the on-effect (e) at various frequencies. Ordinate magnitude of e (logarithmic scale) and on the absis the time (linear scale).

relation between g and the amplitude of the alternating component (viz $V = f+g$) is given in figures 79-A and 80-A for different frequencies and for two animals. The input-output curve is positive and independent of the frequency. At greater amplitude this is not the case. The amount to which the positive tops exceed the R-potential decreases and may even become negative. The two figures represent the two extremes observed. Usually a course such as in fig. 79 is found.

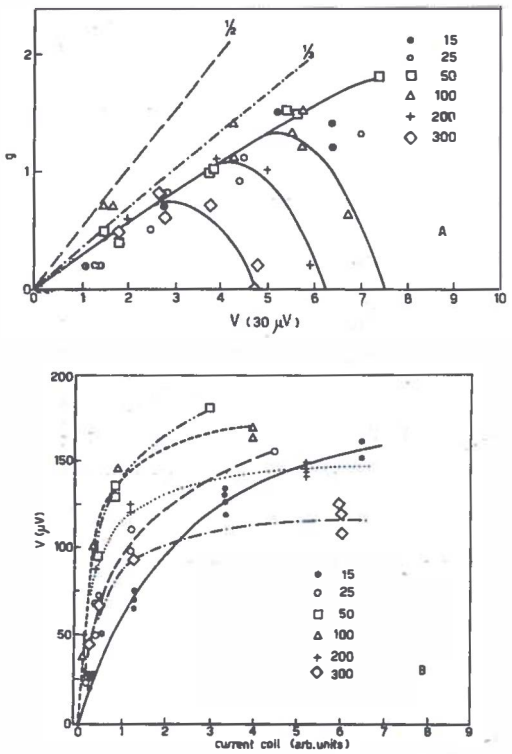


Figure 79. A: The relation between g and V (= f+g) at various frequencies. The lines 1/2 and 1/3 give the relation between g and V if g equals 1/2 and 1/3 V. B: The input-output curve for the same cupula as A.

2.3. The off-effect

When we stop the vibration of the cupula after some time there is always a positive off-effect (h). Imediately after stopping the vibrations there is during a short time a smaller current than in case the cupula has been at rest for a longer time. Up to a certain value the magnitude of the h-peak depends on V, after which further increase of the amplitude of the cupula has no effect. Further h is independent of the frequency of the vibration.

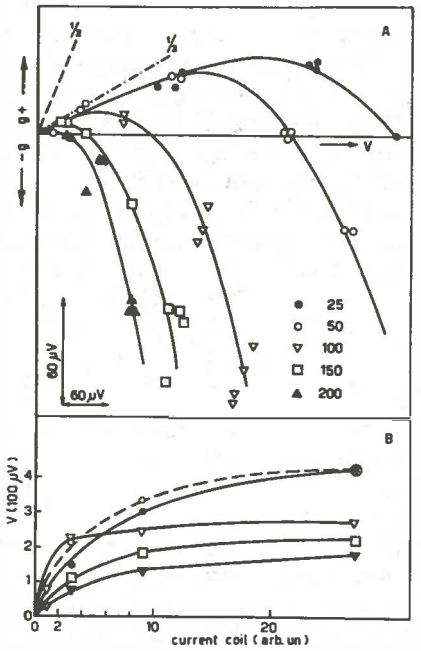


Figure 80. Similar figure as 78 obtained at another animal.

The time-course of h is given in fig. 81. Its halftime (0.08 sec) value is of the same order as found for the fast component of the on-effect.

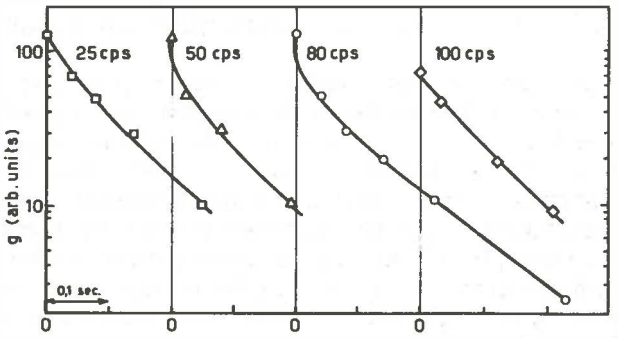


Figure 81. Course of the off-effect; on the ordinate the voltage of h (logarithmic scale and the absis the time (linear scale).

4. Discussion

The main purpose of the experiments described in this

chapter was to demonstrate that the recovery of the resistance of the membrane at the top of the cell (see fig. 29) must be accounted for. The experimental results show that a discussion of the microphonic effect from this point of view will certainly be fruitful. As regards the on-effect however, we might consider this a consequence of small damping of the cupula. If this is the case the halftime value of its decrease must be of the same order of magnitude as found for the amplitude of the vibrations of the cupula when pushed. The order of magnitude of the half time value of the fast component of the off-effect is 0.05 sec. The halftime value of the transient vibrations of an oscillator is $\frac{0.7\ T}{\ln k} = I$ (see chapter V). In our case $T = \frac{1}{\nu_o} = \frac{1}{100}$ sec and I = 0.05 sec. We calculate for the damping factor k about 1.15. Indications for such a small damping of the cupula were not observed. The damping of the oscillations in figure 35 and 55 is more than 2 and mostly a greater value was found.

Hence the on-effect is not caused by the damping of the cupula. For the exponential return of the potential of a cupula displaced by a constant force, a half time value of the same order of magnitude is found as for the fast component of the on-effect. This suggests that both phenomena are caused by the same process.

In chapter VIII we have introduced a slip effect to explain the electrical behaviour during constant displacements of the cupula. De Vries drew the present author's attention to the fact that with such a mechanical effect the experimental results described in this chapter can be explained. We shall not go into the details of this explanation here, because for the moment we prefer a cellular mechanism as a working hypothesis.

As a consequence of the motion of the cupula the membrane at the top of the cell is deformed and this deformation results in the liberation of acetylcholine. As in the case of the motoric-endplate this substance causes a decrease of the membrane resistance. This idea is mainly based on the results obtained with flaxedil. In the former model we tacitly assumed that the microphonic effect depended only on the momentanious pull on the hair. Now we credit to the cells special biological properties. We assume that the alteration induced by the stimulus in the cell are counteracted by special reactions of the cell. For instance the ACh, produced by the deformation of the cell, must be inactivated because otherwise the membrane remains leaky. Moreover it is possible that like in the membranes of nerves and muscles there are two currents, a sodium current inwards and a potassium current outwards.

On account of these considerations it seems therefore plausible to introduce the following double mechanism as a working hypothesis.

1) In consequence of the motion of the cupula the resistance of the cellmembrane is decreased twice each vibration period.
2) The resistance is restored by a mechanism of the sensory cell itself.

The first mechanism will be indicated by C_i (conductivity increasing), the second by C_d (conductivity decreasing).

The difference between the two mechanisms determines the voltage of the microphonic effect.

In the resting cupula we observed a current round the organ (see chapter VII-2). We may account for this by assuming that the C_d mechanism is not capable of "closing" the membrane completely. When the cupula starts vibrating the C_i is increased and when the C_d-mechanism possesses a kind of inertia the on-effect becomes comprehensible. The course of the on-effect shows at higher frequencies a minor dependence on the frequency; at low frequencies however, only the fast component of the course is found (figure 78).

Concerning the mean value of the microphonic potential (M) at the linear part of the input-output curve we observe that it lies at a specific distance from the R-potential, viz. between 1/6 and 1/3 of V. At a certain cupula this value is nearly the same for different frequencies but it varies with different animals (compare figure 79 and 80). We may describe the sitiation in the linear part of the input-output curve as a kind of dynamic equilibrium of the C_i and the C_d-mechanism. The C_i mechanism depends on the motion of the cupula, the C_d system however will depend on metabolic processes of the cells and this will differ with different animals. Such a variability between the animals will be due to the difference in the course of the curves in figures 79-A and 80-A. When at some animals the ac-component of the microphonic potential drops below the R-potential this means that C_i is more increased than C_d. In other words, in this case the membrane resistance is broken to such an extent that the cell is not capable of restoring it after half a vibration period of the cupula, so that a continuous leak exists and a dc-potential is observed beside the ac-potential.

The off-effect (h) will be due to a similar kind of inertia as the on-effect. At the outset of the vibrations the C_i-mechanism stops but now the C_d is at a high level so that the membrane is "closed" better than when it is in the resting organ. It falls exponential to the state of the resting cupula however, probably because the intensity of the C_d-mechanism will depend on that of the C_i.

I grant that this is only a qualitative description of the experimental results by means of two variables. A quantitative description is needed and more experimental support ought

to be given. The double mechanism is merely proposed here as a kind of working hypothesis from which it is possible to continue the analysis of our problem.

A short discussion of this mechanism in relation with the experimental results obtained in the foregoing chapters, is given in the next section.

5. Final discussion on the microphonic effect

On account of the fragmentary results obtained thus far, it is not possible to give a general outline of the mechanism underlying the microphonic effect. Yet I shall now give some of these ideas which form the guiding theory and with this theory new experiments are being designed. Although I emphasize that the following picture is purely speculative, it nevertheless will inform the reader on what I think at present of the problem under discussion.

The movement of the cupula results in a deformation of the cell membrane at the top of the sensory cell, wether this is done by the model of the gliding hair is not essential. As a consequence of the deformation acethylcholine is released. Between the onset of the deformation and the liberation of ACh there might be a latent time, in which period cellular processes occur. This will be the latent time introduced in section 2.2. of chapter VIII for the explanation of the phase behaviour.

ACh produced the decrease of the membrane permeability to cations and will be inactivated by ACh-esterase. This inactivation is according to Nachmannsohn in most other tissues a fast process; at least when we compare it with the period of vibration we are dealing with in the lateral line organ. This means that the effective amount of ACh (and this determines the voltage of the microphonic effect) depends on the velocity of the deformation of the cell. I think that the amplitude of the cupula is essential for the cell and determines the overall amount of ACh produced but that at low frequencies only a minor fraction is effective because the greater part is inactivated by the ACh-esterase. Consequently the velocity of the cupula is essential for the size of the leak in the membrane. This is probably the cause of the better relation between the voltage of the microphonic effect and the velocity of the cupula ($V=k.a.\nu$) than between the voltage and the amplitude ($V=k.a$) (see section 3 of chapter VIII). Moreover on account of this assumption the effective amount, nearly in phase with the velocity of the cupula, is ahead of the displacement of the cupula and therefore at low frequencies the phase of the microphonic effect is in phase ahead of the displacement of the cupula.

I think that with this conception the mechanical slip explanation for the phase behaviour has become superfluous. Certainly there is a mechanical slip but this is a slow process which is only essential at constant displacements of the cupula. The rapid fall of the d. c. -potential at constant displacements is not a slip effect but indicates the inactivation of ACh.

Apart from some questions of minor importance at the moment, there remains one difficult problem, viz. the superposition effect. The microphonic effect of a high frequency vibration is amplified in the negative peak of a low vibration on which it is superimposed. See section 5 of chapter VII and figure 46. This effect is very pronounced in the linear part of the input-output curve. We could not explain this effect with the deformation model because we assume this to be of a linear character whereas the superposition effect is a nonlinear reaction.

Though not worked out in detail the double mechanism proposed now seems to offer a possibility to give an explanation of this remarkable effect. The superimposed vibration possibly disturbs the dynamic equilibrium between the conductivity increasing (ACh) mechanism and the conductivity decreasing system (ACh-esterase) belonging to the basic vibration.

I think we are now in a similar situation as in 1951 when de Vries formulated his hair-hypothesis. This idea was then a valuable working hypothesis – at least to me – and I can only hope the same will be the case with the double mechanism proposed here.

Chapter XIII

FUNCTION OF THE LATERAL LINE ORGAN

Introduction

In literature there is some controversy about the function of the lateral line organ of fish. It is maintained on the grounds of electrophysiological investigations that the organ is a vibration detector, with which the animal perceived vibrations of low frequency in the water (Pumphrey). Others – and one of the most prominent investigators of this organ, Dijkgraaf, in particular – are of the opinion that static deformations are the more biologically adequate stimuli for this organ. The latter opinion is mainly based on the fact that so far nobody has been able to train fish to vibrations applied to the lateral line organ.

In the electrophysiological experiments described in previous chapters, vibrations are applied to the organ and it is therefore important to know whether these are of biological significance to the animal.

The first part of this chapter will discuss experiments in which the conditioned reflex method was used to investigate whether the ruff (Acerina cernua) could perceive vibrations with the lateral line organ. The second section will deal with some considerations of the function of this organ for the fish.

I. THE TRAINING OF RUFFS TO VIBRATIONS OF THE LATERAL LINE ORGAN

The aim of the experiments described here was twofold.

In the first place I wanted to show that these fishes are able to perceive vibrations of a low frequency with the aid of the lateral line organ. In these experiments I had to be sure that other sense organs, especially the labyrinth and the sense of touch of the skin, were not stimulated.

Secondly I wished to see whether the curve for the frequency sensitivity of the animal has the same course as the equal response curve of the microphonic effect (fig. 37).

Pending the inquiry of the microphonic effect we have used the equal electrical response of the lateral line organ to study the influence of the frequency. For the entire animal we can only use an observable change of its behaviour. One method is to interest the animal in the stimulus by feeding it during

the stimulation of the investigated organ. After some time it shows a typical "search"-behaviour when the stimulus is applied only (conditioned reflex). A strength of the stimulus "only just" perceptible can be used as the equal response in this case. Such a technique is used for example by von Frisch and Stetter (1932).

1. Methods

The animal was put in a round glass vessel (diameter 25 cm) which was placed above a big coil. A diagram of the experimental situation is given in fig. 82. The resistance of the coil was 1 ohm and the coil consisted of 400 windings. With an R. C. generator and an amplifier an alternating current of a given frequency was led through the coil. On the skin of the canalis supra orbitalis a magnet was mounted as indicated in fig. 82. When the openings in the skin were made as small as possible and the magnet was "clinched" to the skin then it remained on the fish, packed in the muscus of the skin, for several months. The magnetic axis was perpendicular to the surface of the skin. When thus orientated it was moved by the gradient of the magnetic field of the coil. Several other more effective arrangements had been used in the beginning. They were all abortive as in a few days the magnet became detached.

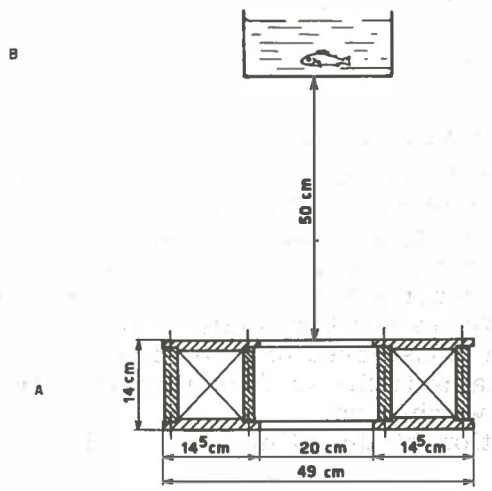


Figure 82. Experimental arrangement in the training experiments. A is the coil (diameter 49 and central opening 20 cm) B is the vessel with the fish.

As a consequence of the vibration of the skin the fluid in the canal oscillates and together with this the cupula. So the

driving method is essentially the same as in the electrophysiological experiments.

Acerina can be easily trained to optical stimuli. To avoid undesirable optical conditioned reflexes we have used blinded animals. The eyes were removed when the animal was under an ethylurethan narcosis.

First the fish learned to take food from a small glass hook. A "domesticated" fish learned this in 5 to 10 feedings. The next step was to apply a vibration of the lateral line organ during the feeding. The reaction of the animal was observed after the onset of the vibration.

2. Results

2.1. *Spontaneous reactions to the vibration of the magnet on the skin*

A ruff, not trained before, with a magnet on the skin of the lateral line organ on top of the head sometimes shows the following reactions when it moves in an alternating magnetic field. When the stimulus is weak the animal stops moving and rises to the surface.

On strong stimuli the animal moves backwards and demonstrates an alarmed behaviour. If it is stimulated for a longer time, say a minute, it tries to remove the magnet by brushing against the wall of the aquarium. A similar behaviour is seen when a parasite is on the skin of the head.

2.2. *Preliminary measurements*

The first animal taught to take food from a small glass hook learned this in about 10 feedings. In the next stage the lateral line organ was stimulated magnetically for about half a minute, after which the animal was fed. With this animal clearly positive reactions were obtained after about 20 feedings (5 a day). We trained 6 animals in all.

Reactions were considered to be positive when:

a. The animal came to the surface after the current through the coil was switched on.
b. After application of the stimulus it made snapping movements.

A well trained hungry animal nearly always demonstrated this behaviour after the onset of the stimulus.

In a number of preliminary experiments I diminished the current until I got no reaction. Although the values obtained were not quite exact, they showed a tendency to group around the equal response curve of the microphonic effect of the cu-

pula; On account of these results it seemed worth while to make quantitative threshold measurements.

2.3. *Quantitative measurements*

2.3.1. *Procedure.* The vessel was placed at such a distance from the coil that horizontal and vertical variations of the magnetic field in this vessel could be neglected. So the stimulus to the fish was independent of the place of the animal in the vessel.

To avoid an onset effect of the current this was shut by an electronic shutter. In 1 second the current increased from zero to a given value. We measured the voltage on the coil and calculated the current by means of the impedance. When starting the experiments we first drew the animal's attention to the stimulus by a fairly strong one. When starting with a weak stimulus we got no reaction, whereas after a number of stronger ones the same weak stimulus gave 100% positive reaction.

A number of fixed values of the current was taken and for each value the reactions of the animal were investigated.

The order of the strength of the stimuli was arranged by a coworker. The observer (the present author) had not the slightest idea about this during the experiment, so that his interpretation of the behaviour of the animal could not be influenced. It was proved that, when the observer applied the stimulus, his interpretation was indeed very subjective: The observer said when a stimulus could be applied and then he got ten seconds to tell whether the reaction was positive or negative.

In case he observed a positive reaction the animal was fed, except in those cases where no current passed the coil; then the coworker prevented the feeding. Each time the animal received only one Tubifec worm.

In 1 - 2 hours we investigated one frequency and not more than two or three a day. Then the observer was "saturated", not the animal.

2.3.2. *Results quantitative measurements*

In fig. 83 the percentage of positive reactions was plotted against the current through the driving coil. There was always a "zero-effect" (a positive reaction when there was no current through the coil). For this effect the values obtained were corrected by using

$$A = B + (1 - B)Z$$

In this formula A is the observed value, Z the zero-effect and B the real fraction. One comes to this formula on account of the following considerations (see de Vries 1950). No reac-

tion occurs in the fraction (1 - B). However, the zero effect really implies that a fraction (1 - B)Z is followed by a positive reaction of the animal. Hence the total number of positive reactions equal B + (1 - B)Z. In fig. 83, B is plotted against the current. The current giving 50% positive reaction was taken as threshold value and this value was plotted against the frequency in fig. 84 for the three animals we studied.

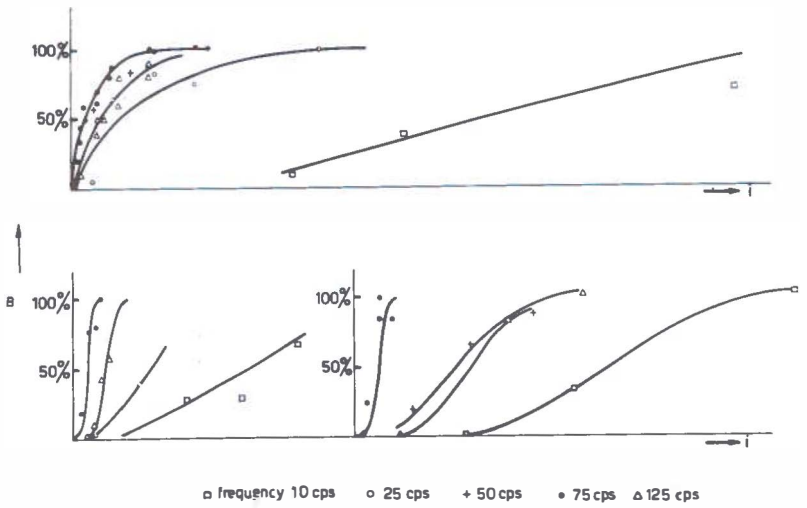


Figure 83. Experimental results obtained in the quantitative training experiments. On the basis the current through the coil and on the ordinate the number of positive reactions after correction for the "zero effect".

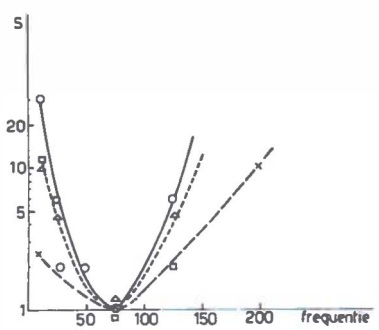


Figure 84. The threshold curves for three fishes. S is the current through the coil normalised in such a manner that the current to obtain 50% positive reaction at 75 c.p.s. is 1.

We had to make certain that we were here indeed concerned

with stimulation of the lateral line organ and not with a tactile stimulus to the skin or with a stimulation of the labyrinth. I therefore cut the nerve to the cupulae which were stimulated. It was impossible to obtain a reaction from the trained fish even when the current was ten times stronger than the strongest current used in the normal situation. Next the magnet was put on the skin of the intact canal at the other side of the head. Now it was possible to get marked positive reactions at currents we also used before, but now without new training. Hence we are sure that no sense organs are involved other than the lateral line organ.

3. Discussion

From the results described in the foregoing part I thought it permissible to draw the conclusion that the ruff (Acerina cernua) can perceive a low frequency vibration of its lateral line organ. Furthermore, since the threshold curve agrees with the equal response curve of the microphonic effect, this effect gives a good idea of the potentiality of the lateral line organ. At higher frequencies the threshold is relatively higher than would be expected from the electric activity. This may be caused by the nerve fibres, which are incapable of following the higher frequencies, or by the central nervous system, which may be less sensible to the higher frequencies. The former seems not so probable at the moment, for most nerve fibres can follow the whole frequency range used here. Whether this is the case with the lateral line nerves has not been investigated.

The minimum threshold of each sense organ is determined by the Brownian motion (see for an extensive discussion de Vries (1956) and his former publications on this subject). A discussion of the threshold in relation with the Brownian motion gives an interesting result. For this discussion we need the theoretical minimum threshold displacement of the cupula determined by the Brownian motion and the displacement of the cupula at threshold stimulation.

We calculate the theoretical minimum in the following way. When the cupula vibrates with the minimum perceptible amplitude (a), the energy dissipated per sec is $\frac{1}{2} b (2\pi\nu)^2 a^2$. The friction b can be calculated because the system is about critically damped (see chapter V); then $b = mf$. The mass m is about 0,4 mg and f is of the order of 100 dynes/cm; we obtain for $b = 0{,}2$ c. g. s. units.

There will be a minimum time during which the stimulus must be maintained to be perceptible for the animal. I have not measured this time exactly but think it is not much shorter than 0,5 sec. Now we may state the energy applied may not

be smaller than the thermal agitation of a single molecule 1 kT (4.10^{-14} ergs). If this is not the case the sensorium will be continuously stimulated by the Brownian motion and the "signal" becomes submerged in the "noise". Hence we suppose

$$0{,}5 \tfrac{1}{2} b (2\pi\nu)^2 a^2 = 4.10^{-14}$$

in the case ν = 75 c.p.s. This is the theoretical minimum perceptible amplitude and calculated for $a = 0.3.10^{-8}$ cm.

The real threshold amplitude of the cupula in the closed canal is unknown. But we have an idea of the order of magnitude when we know the displacement of the skin. At the distance from the coil where the fish was kept in the training experiments the amplitude of the skin could not be measured. Therefore the fish (tied) was put immediately above the coil. The double amplitude of the skin was measured in a way similar to that described for the free cupula in chapter II. (In this case the microscope was approximately horizontal). At a current 25 times the threshold strength the amplitude of the skin was 2,5 micron. In the training experiments the force acting on the magnet was smaller, because the animal was at a greater distance from the coil. The gradient of the field was determined by measuring the force acting on a magnet (orientated in a similar way as the magnet on the fish) at various distances from the coil. In this way we found a correction factor 40. Hence the real amplitude of the skin is 25 x 40 weaker, so 2,5 millimicron or 25 Angström. This is about 10 times the calculated theoretical minimum.

It must be emphasized that this value is not exact, because both the correction factor and the amplitude were only roughly estimated. Moreover an error may arise from the fact that with increasing distance from the coil, the gradient of the magnetic field decreases faster than the field itself. So it could happen that the couple of force acting on the magnet gave some extra effect in the actual situation the threshold was determined. Therefore the agreement between the experimentally obtained value and the theoretically calculated is better then we expected. This result of the discussion supports our statement that we are really dealing with the threshold of the lateral line organ.

II. FUNCTION OF THE LATERAL LINE ORGAN

There are several publications in which the function of this lateral line organ is discussed (for references see Dijkgraaf). In a more recent paper Pumphrey (1950) started from the idea that in aquatic animals the discontinuity between the surface of the body and the surrounding water is less abrupt than it is for an animal in the air. This means that a body moving

through the water close to the surface of the animal "will deform that surface to an extent comparable with the deformation by bare contact". The lateral line organ can be considered a specific organ to detect these deformations. This idea is as far as I can see in full agreement with Dijkgraaf's observations and ideas on this organ. He, and before him several other investigators, proved that fishes and some amphibia are able to localize the position of a moving object with the aid of the lateral line organ.

In this section the ideas of Dijkgraaf and Pumphrey are developed in more detail from an other point of view.

1. Theoretical consideration about the function of the organ

To avoid a long discussion I shall first describe what happens at the surface of the body of a fish when the animal moves through the water, or the symmetrical case, a resting fish in water currents.

We start from the model given in fig. 85. This is a stream-

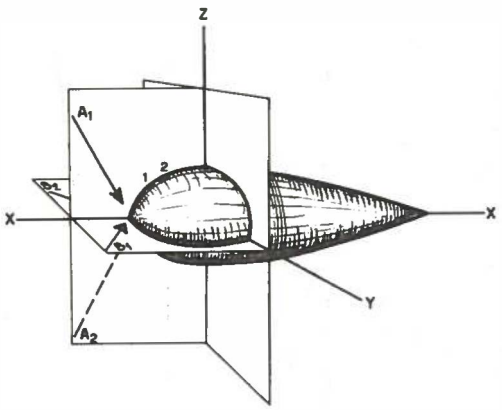


Figure 85. Diagram indicating the directions from which the model can be attacked by a current. The measuring points used in the calculations of figure 87 are indicated by 1 and 2.

line form, like the body of the fish. When the body is attacked by a water current the pressure on the surface of the body varies from point to point. We take two points corresponding with the most cranial and the most caudal point of the canal on top of the head of the animal (see fig. 85). The pressure on the various points on the body alters with the angle of attack (= angle between the direction of the current and the spindle of the body). When the model is caught from A_1 the measuring points are on the pressure side whereas when the stream comes from A_2, they are on the suction side.

Whether the current comes from the right or from the left, the difference in pressure at points 1 and 2 is the same if the angles of attack are equal.

Of importance is the course of the difference in pressure at points 1 and 2 in relation to the direction of the current. The problems we have here, are similar to those met with in aerodynamics; they are there studied in detail. These data can be used in our consideration of the model of the fish, provided we are below velocities in which eddy currents occur. The course of the pressure on a fuselage form is given in fig. 86. The data used in fig. 86 are taken from a report of de Lange (1948). We see in this figure that the steepness of the curve indicating the change of pressure along the body is highest at the front of the body. Moreover the course of the curve alters when the direction of the current is changed. From these figures we calculated the difference in pressure for points 1 and 2 of fig. 85 in relation to the direction of the current. This difference is given in fig. 87. It will be clear that the difference in pressure on points 1 and 2 depends on the direction of the current. When the body is attacked by alternating water currents the pressure at the points alternates.

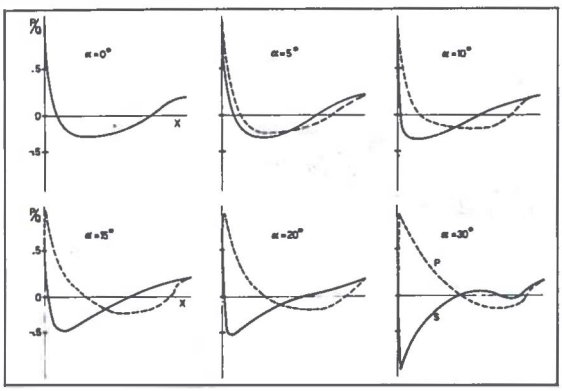


Figure 86. The change in pressure on the model of figure 85. X the place on the longitudinal axis of the model. P the observed pressure and $Q = \frac{1}{2}\rho v^2$ (ρ specific weight of the fluid and v the velocity of the fluid) α is the angle between the direction of the current and the X-axis in the XZ-plane of figure 85. The full drawn curve in the case α is positive (measuring points on the pressure side); the dotted line for α negative (points on the suction side).

We shall now look at fig. 10, which gives the distribution of the lateral line organ over the body of the fish. We see that this organ is especially developed at the head; hence at the very same place the pressure gradient is greatest. This means that, when the lateral line organ is capable of detect-

ing a difference in pressure, this organ will enable the fish to detect the direction of the current.

When a fish is hunting it will fix its prey with its eyes. However, the binocular field of vision is rather small at these animals with lateral eyes, and therefore the optic localization rather poor.

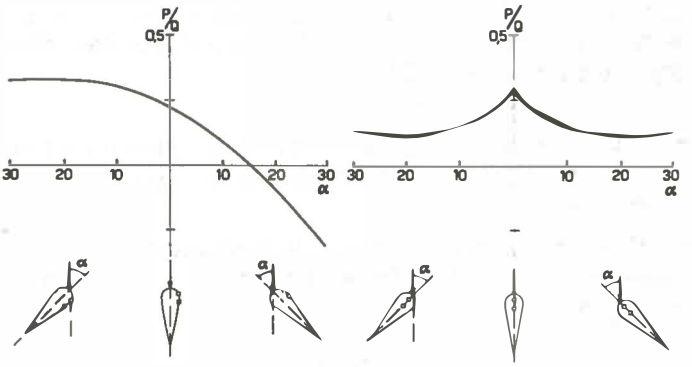


Figure 87 The relation between the angle of attack (α) and the change of the difference in pressure between the two points indicated in figure 85.

No exact values are available on the visual fields of the animals. A study of this is in progress. I think we have a situation such as schematically indicated in fig. 88. The overlapping of the fields of the eyes seems to be rather small. It seems quite possible that at a distance of about 5 - 10 cm in front of the head, the animal experiences difficulties with the optical localization of its prey. Usually the prey will be moving and in doing so produce alternating water currents. We may say that the fish is caught by an alternating water current. Consequently the pressure on the head varies.

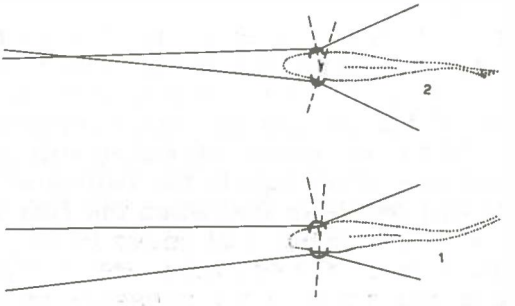


Figure 88. Visual field of Acerina. In 1 eyes in the normal position and in 2 maximal forward rotated eyes. The overlapping of the visual fields may be more than indicated in 2. The drawings are made from photographs taken during the feeding of the fish.

The idea is that with the lateral line organ the animal perceives these variations. The difference in pressure at both sides of the bone bridge varies, which produces an alternating movement of the skin and of the fluid in the canal and so the cupula is moved. When the animal "knows" the stimulation pattern for different direction of a current it will be able to localize its prey with the lateral line organ.

In animals with free organs the cupula will be moved by the alternating water movements themselves, as has already been stated by Pumphrey (1952).

2. Experimental support to the theoretical considerations on the function of the lateral line organ

From the above mentioned point of view we shall now discuss observations taken from literature and some made by the author himself.

2.1. *The detection of non-moving objects*

As early as 1933 and in later publications Dijkgraaf stated that the organ should be regarded as an organ for distance sense touch (Organ für "Ferntastsinn"). According to this author the animal notices with this organ moving as well as non-moving objects in the water.

He reports that a blinded Corvina did not bump against the wall of the aquarium when the animal moved slowly through the water. At about 1-2 cm from the wall the fish turned away.

Dijkgraaf explained this by assuming that the fish perceived with the lateral line organ the change in resistance it experienced while approaching the aquarium wall. We shall describe how this organ might be stimulated with the terminology used above.

Considering the forces acting on the fish we have the same situation whether the fish moves or the water. Around a moving fish is a current pattern and this pattern is determined by the velocity of the animal and the curvature of the body. In front of the head the water particles will separate. This happens at a distance which equals the radius of the curvature of the head. It will be clear that when the fish approaches an obstacle the water particles will move in an other way than at large distance from the obstacle. Hence the current pattern changes and consequently the pressure on the skin of the lateral line organ. This means that the cupula is displaced when the animal approaches an obstacle. This problem was studied once more at the ruff.

A blinded ruff was placed in a round aquarium (diameter 25

cm). The aquarium was divided by glass bars into two parts. Apart from direct contact, the animal proved to be absolutely unable to perceive these bars (diameter 0,3 cm). Again and again it bumped against them. In another experiment a glass plate was placed vertically in the vessel. This too, the animal was unable to notice, whatever its velocity. Only when the animal was almost at rest I got the impression that it observed the obstacle at a distance of not more than a few millimetres. But this may be caused by a tactile stimulus to the skin. So far I have not found any indication that the ruff is able to perceive non moving objects with the lateral line organ.

According to Dijkgraaf (personal communication) the lateral line organ of Corvina is even more developed than that of Acerina. This might be the cause of the difference in our investigations.

2.2. *Detection of moving objects with the lateral line organ*

Dijkgraaf (1949, see also his other publications) e.g. observed that blinded Gobii paganellus were able to localize other blinded and normal animals. Concerning the courtship "behaviour", there was no difference between blinded and normal animals. Though he did not describe controlling experiments it seemed very probable that the lateral line organ was prominent in this behaviour.

Moreover there are some older observations stating that a blind pike can catch its prey in the normal way, but not when the lateral line organ is destroyed (see Dijkgraaf (1933)).

Several times Dijkgraaf described how at a distance of 2 to 3 cm blinded fishes are perfectly able to localize a thin feeding bar. We observed that such a bar produces a microphonic effect of the lateral line organ when the bar is held in the hand in the same way as Dijkgraaf did in his training experiments. Due to slight involuntary movements of the hand the frequency and the amplitude of the microphonic effect is variable. Consequently it seems certain that the water displacements produced by the vibrating bar are effective for the lateral line organ.

At the ruff the following additional observations were made. Here, too, the blinded fish is very well able to localize a similar feeding bar as Dijkgraaf used. In the training experiments described in section 1 it rarely snaps amiss the feeding bar. When the same bar is resting on the bottom of the vessel the animal does not show any interest in it. The same is the case when the feeding bar is fixed to a support and hangs in the water. But as soon as we take it in our hand the blinded fish perceives it at 5 cm distance and snaps at the end, whether there is food on it or not.

In some blinded animals the nerves from the cupulae of the canalis supra orbitalis at one side of the animal had been cut. These animals also showed an immediate reaction to the bar but they missed the bar when they snapped at it. After about ten feedings they once more localized the bar as well as before. Apparently they had learned to correct the loss of part of the organ. These results indicate that the lateral line organ is involved in the localization of moving objects in the manner above.

Conclusion

From the experimental results described in this chapter we think we may state:

1. The frequency characteristic of the microphonic effect gives us a good idea about the potentiality of the lateral line organ because the "threshold curve" has a similar course as the equal response curves of the microphonic effect. It might have been possible that the afferent nerves are stimulated not electrically but in a chemical manner. If this was the case we should expect that the "threshold curve" has an other course than the frequency characteristic obtained for the microphonic effect. Since this is not the case the microphonic effect can be considered a good measure for the cellular processes induced by the stimulus.
2. On account of the absence of a microphonic effect at constant displacements of the cupula and the fairly close agreement between the electrophysiological and the training equal response curves we expect that constant displacements give no nerve activity other than that at the onset and offset of the displacement. This has not been investigated by us so far. However, the results obtained by Sand (1937) and Dijkgraaf and Elbers (personal communication) are not in agreement with this statement. This question will be studied. As for free cupula, it seems more probable that they are sensitive to vibrations than to constant displacements. Otherwise, the lateral line organ would be continuously stimulated by the movement of the animal itself.
3. The lateral line organ on the head of Acerina cernua is a tactile sense organ enabling this fish to localize moving objects in its vicinity. The alternating water currents produced by the object are the stimuli for the organ. The lateral line organ seems to be a sense organ with which the fish can localize its prey at short distances.
4. On account of theoretical considerations it might be possible that some species will be able to localize a non-moving object with the lateral line organ. We have no observations that Acerina is capable of doing this. Dijkgraaf observed a behaviour of Corbina pointing in this direction. A more detailed study of this animal seems worth-while.

SUMMARY

In 1952 de Vries formulated a hypothesis – the hairhypothesis – by means of which it was possible to give a qualitative description of most of the then known microphonic effects of the labyrinth organs and the lateral line organ. A new effect, the superposition effect was discovered by him as a consequence of this hypothesis.

According to this theory the microphonic effect is determined by a force acting in the longitudinal direction of the hair at the top of the sense cell (see Chapter VI). This notion is the starting-point of the present investigation.

The structure of the lateral line organ is investigated and compared with structures in the labyrinth (Chapter III). The pear-shaped sense cells of these organs show a clear resemblance. On the thickened membrane at the top of the cell one hair is found with the lateral line organ and several with the labyrinth organs. These hairs are probably perpendicular to the cell surface. The gelatinous cupula should be conceived as a secreting product of the supporting cells. These cells form in addition a mechanical solid foundation for the sense cells.

A brief and therefore incomplete discussion of the literature on the microphonic effect of the labyrinth organs makes it clear that the electric effect from these organs is complicated. It consists of a primary sense effect and two secondary potentials originating from ganglia. The latter are lacking with the lateral line organ. On these grounds and because of the greater experimental possibilities, the lateral line organ is to be preferred to the labyrinth for the study of the microphonic effect.

An investigation into the mechanics of the lateral line organ demonstrated that a displacement of the entire organ over the sense epithelium was effective for the microphonic effect. In a situation in which the organ approximates the natural situation very closely, the amplitude is small and mostly not to be measured. Even when the microphonic effect has reached its saturation value, the amplitude of the cupula is smaller than one micron. The cupula behaves plastically to constant and elastically to oscillating forces. On account of this the organ is an ideal vibration perceptor. Similar properties had already been found by von Békésy for the tectorial membrane. Mechanically the cupula behaves to vibrations as a simple harmonic oscillator which is (approximately) critically damped.

The hair hypothesis is discussed at length in Chapter VI. The voltage of the microphonic effect is assumed to be proportional to the pull at the hair, and from this point of view a number of problems are posited.

An important part of the experimental results is inconsistent with this starting-point (Chapter VII). Essential is that at small amplitudes, the voltage of the microphonic effect is proportional to the amplitude of the cupula. However, in the same range of amplitudes of the cupula a non linear effect occurs (superposition effect Fig. 46) in the case of a compound vibration. With a simple mechanical model it is not possible to explain this effect.

At constant displacements no permanent increase of the direct current potential (already present in the resting cupula) could be measured. This phenomenon can be explained by means of a slip-effect (Chapter VIII-3).

It was demonstrated that at a constant amplitude of the cupula the voltage of the microphonic effect decreases at low frequencies. At constant velocity of the cupula the microphonic effect is much less dependent on the frequency (see Chapter VIII-3.2). So apparently the velocity is more essential than the amplitude for the origination of the microphonic effect. The slip process is probably too slow to account for the fact that at low frequencies only a small percentage of the amplitude is effective for the microphonic potential (see VIII-3).

The phase behaviour of the microphonic effect of a vibrating cupula deviates from the phase of the mechanical response (figs. 43, 44 and 45). A plausible explanation for this can be given with the aid of a latent time (see VIII-2.2).

Since the deviations of the cupula proved to be very slight ($< 1\mu$) the sense cells – if the hair hypothesis was correct – would be stimulated ineffectively, at least in the case of a perpendicular position of the sense hairs (ca 7 μ long). Energetic considerations rendered it unlikely that a force in the longitudinal direction of the hair is essential for the sense cell (page 102).

A large part of the experimental results can be described by a new model. In this the hairs merely serve to increase the friction between the sliding surfaces. The same result can be obtained however, when the hairs are assumed to be inclined and then pulled. But the latter model is less likely on account of the histological investigations, in which the sense hairs were found to be perpendicular.
Neither model accounts for the superposition effect.

The problem is next approached from a more physiological view-point. Of central interest are here the processes occurring in the sense cell. In this connection a survey of the

literature on membrane potentials of nerve and muscle is given in chapter IX. Proceeding from processes known in these organs it is now endeavoured to arrive at a new basis for experimental research.

It was proved that at the lateral line organ, just as in the case of the labyrinth organs the bloodsupply, pharmaca, ions and the temperature influence the voltage of the microphonic potential.

Acethylcholine is likely to play an important part in the origin of the microphonic effect of the lateral line organ (Chapter XI).

In view of this a double mechanism is proposed in Chapter XII as a provisional working hypothesis.

Acethylcholine is formed as a consequence of the mechanical deformation of the top of the sense cell. This gives rise to a local change in the permeability and consequently to a current round the sense cell (see model in figure 29). This current is observed in the form of the microphonic potential. However, the cell possesses a reaction mechanism which rapidly inactivates the liberated acethylcholine. According to this notion it is probable that the effective quantity of the acethylcholine depends on the rate at which it is produced, so on the velocity of the cupula. Possibly this is the cause of the influence of the frequency (p. 97) on the voltage of the microphonic effect.

With the aid of this hypothesis an attempt is being made at present to reanalyse the superposition effect.

The final chapter discusses some biological experiments concerning the function of the lateral line organ. We succeeded in training the fish, which was also used in the electrophysical investigations, to vibrations of the lateral line organ. Attempts of previous investigators had hitherto failed. It was demonstrated that the frequency characteristic of the microphonic effect is a good criterion as to what the animal can perceive with this organ. The organ is probably used to localise a prey at a short distance.

SAMENVATTING

In 1952 stelde de Vries een hypothese op – de haren hypothese – waarmee het mogelijk was een qualitatieve beschrijving te geven van de meeste toen bekende microphonische effecten van de labyrinth organen en het zijlijn orgaan. Een nieuw effect, het superpositie effect, werd door hem ontdekt als een consequentie van de hypothese.

Volgens deze theorie is een kracht, werkend in de lengte richting van de haar op de zintuigcel, bepalend voor het microphonisch effect (zie Hoofdstuk VI). Dit idee vormde het uitgangspunt van het hier beschreven onderzoek.

De bouw van het zijlijn orgaan werd onderzocht en vergeleken met structuren in het labyrinth (hoofdstuk III). De peervormige zintuigcellen van deze organen vertonen duidelijke overeenkomst. Op het verdikte membraan aan de top van de cel zit bij het zijlijn orgaan één en bij de labyrinth organen meerdere haren. Deze haren staan waarschijnlijk loodrecht op het cel oppervlak. De gelatineuse cupula moet opgevat worden als een afscheidingsproduct van de steuncellen. Deze cellen vormen bovendien een mechanisch stevige onderlaag voor de zintuigcellen.

Een korte en daarom onvolledige bespreking van de literatuur over het microphonisch effect van de labyrinth organen maakt duidelijk dat het hiervan afgeleide electrisch effect gecompliceerd is. Het bestaat uit een primair zintuigeffect en twee secundaire potentialen afkomstig van ganglia. Deze laatste ontbreken bij het zijlijnorgaan. Om deze reden en om de grotere experimentele mogelijkheden verdient het zijlijn orgaan voor het bestuderen van het microphonisch effect de voorkeur boven het labyrinth.

Een onderzoek naar de mechanica van het zijlijn orgaan wees uit dat een verplaatsing van het gehele orgaan over het zintuigepitheel effectief was voor het microphonisch effect. In een situatie waarin het orgaan de natuurlijke toestand zeer nabij komt, is de amplitudo van de cupula zeer klein en meestal niet te meten. Zelfs wanneer het microphonisch effect reeds zijn verzadigingswaarde bereikt heeft is de amplitudo van de cupula kleiner dan één micron. De cupula gedraagt zich plastisch voor constante en elastisch voor oscillerende krachten. Hierdoor is dit orgaan een ideale trillingsperceptor. Zelfde eigenschappen waren reeds door Békésy voor het tectorial membraan gevonden. Voor trillingen gedraagt de cupula zich mechanisch als een enkelvoudige harmonische oscillator die (ongeveer) critisch gedempt is.

Met behulp van de, in hoofdstuk VI uitgebreid besproken, harenhypothese worden een aantal experimenteel te benaderen problemen gesteld. Aangenomen wordt dat het voltage van het microphonisch effect evenredig is met de trek aan de haar. Een belangrijk gedeelte van de in hoofdstuk VII besproken experimentele resultaten zijn niet in overeenstemming met dit idee.

Essentieel is dat bij kleine amplituden de spanning van het microphonisch effect evenredig is met de amplitudo van de cupula maar dat bij een samengestelde trillingsvorm in het zelfde amplitudo gebied een niet lineair effect optreedt (superpositie effect). Met een eenvoudige model-voorstelling is het niet mogelijk hiervoor een verklaring te geven.

Bij constante verplaatsingen kon geen blijvende vergroting van de gelijkstroom potentiaal (in de stilstaande cupula reeds aanwezig), gemeten worden. Met behulp van een slipeffect is dit verschijnsel te verklaren (VIII-1). Het slipeffect is waarschijnlijk te traag om te kunnen verklaren dat bij lage frequenties slechts een klein percentage van de amplitudo effectief is voor het microphonisch effect (zie VIII-3). Gevonden werd dat bij constante amplitudo van de cupula het voltage van het microphonisch effect bij lage frequenties afneemt. Bij constante snelheid van de cupula is het microphonisch effect veel minder afhankelijk van de frequentie (zie VIII-3.2). Klaarblijkelijk speelt voor het microphonisch effect de snelheid dus een belangrijker rol dan de amplitudo.

Het phase gedrag van het microphonisch effect van een trillende cupula wijkt af van de phase van de mechanische responsie (zie fig. 43, 44 en 45). Met behulp van een latentie tijd kan hiervoor een plausibele verklaring gegeven worden (zie VIII-2.2).

Daar bleek dat de uitwijkingen zeer klein zijn ($< 1\mu$) zou bij loodrechte stand van de zintuigharen (ca. 7 μ lang), indien de haren hypothese juist was, de zintuigcellen op een ineffectieve wijze geprikkeld worden. E en energetische beschouwing maakte onwaarschijnlijk dat een kracht in de lengte richting van de haar essentieel is voor de zintuigcel (pagina 102). Met een nieuw model, waarbij de haren rechtopstaan en alleen dienen om de wrijving tussen de glijdende oppervlakken te vergroten, zijn een groot deel van de experimentele resultaten te beschrijven. Het zelfde gelukt echter ook wanneer aangenomen wordt dat de haren scheef staan en evenals in het oude model een kracht in de lengterichting van de haar effectief is. Dit model is naar aanleiding van het histologisch onderzoek, waarbij men vond dat de zintuigharen rechtop staan minder waarschijnlijk. Beide modellen geven geen verklaring voor het superpositie effect.

Het probleem wordt nu van meer physiologische zijde be-

naderd. Hierbij staan de processen welke optreden in de zintuigcel centraal. In verband hiermede wordt in hoofdstuk IX een overzicht gegeven van de literatuur over membraan potentialen van zenuw en spier. Uitgaande van bij deze organen bekende processen wordt thans getracht tot een nieuwe basis voor experimenteel onderzoek te komen. Gebleken is dat evenals bij de labyrinth organen ook bij het zijlijn orgaan de bloedvoorziening, pharmaca, ionen en de temperatuur een invloed uitoefenen op de grootte van het microphonisch effect (hoofdstuk X).

Waarschijnlijk speelt acethylcholine een belangrijke rol bij het ontstaan van het microphonisch effect bij het zijlijn orgaan (XI). Naar aanleiding hiervan wordt als voorlopige werkhypothese in hoofdstuk XII een dubbel mechanisme voorgesteld. Tengevolge van de mechanische deformatie van de top van de zintuigcel wordt acethylcholine gevormd. Hierdoor treedt een locale permeabiliteits verandering op en loopt er om de cel een stroom (zie model in fig. 29) en deze stroom wordt waargenomen in de vorm van de microphonische potentiaal. De cel beschikt evenwel over een reactiemechanisme wat de gevormde acethylcholine snel inactiveert. De effectieve hoeveelheid ACh hangt dus tenslotte af van de snelheid waarmede ACh geproduceerd wordt. Het is mogelijk dat de invloed van frequentie (pag. 97) op het voltage van het microphonisch effect hierdoor wordt veroorzaakt.

Getracht wordt met behulp van deze onderstelling het superpositie effect opnieuw te analyseren.

Het laatste hoofdstuk bespreekt enige biologische proeven over de functie van het zijlijn orgaan. Het gelukte de, bij het electrophysiologisch onderzoek ook gebruikte, vissoort te dresseren op trillingen van het zijlijn orgaan. Men was hier tot nu toe nog niet in geslaagd. Aangetoond werd dat de frequentie karakteristiek van het microphonisch effect een goede maatstaf is voor hetgeen het dier met dit orgaan kan waarnemen. Het dient waarschijnlijk voor het localiseren van een prooi op korte afstand.

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